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THE OPTIMUM PH FOR DIASTASE OF MALT ACTIVITY

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The researches of Sherman et al. (1919) are usually referred to (Meyer and and Anderson, 1952) whenever the optimum hydrogen ion concentration for diastase is considered. By using diastase which had been extracted and purified in the laboratory, Sherman et al. found the optimum pH to be about 4.5. In our experiments with diastase of malt powder, which was secured through a supply house, we obtained a hydrogen ion activity curve markedly different from the one reported by Sherman et al. for amylase of malt. One is led to believe that Sherman et al. actually obtained a curve for β-amylase rather than diastase, inasmuch as the curves resulting from the use of β-amylase are similar to the curves presented by Sherman et al. Therefore, it would seem desirable to report the hydrogen ion activity curve of commercially available diastase of malt. In addition, hydrogen ion activity curves determined for α-amylase and β-amylase are presented herewith.

METHODS AND MATERIALS

Diastase of malt U.S.P. IX was used in all experiments involving diastase. A one-half percent solution of diastase was prepared in distilled water and allowed to stand for one hour. Next, it was suction-filtered through a gooch filter lined with acid-washed gooch asbestos. The filtration removed starch grains present in the crude diastase of malt preparation, thereby adding to the reliability of the tests used to determine the amount and rate of diastase activity. A fresh solution of diastase was prepared each day.

Alpha-amylase and β-amylase were purchased from Nutritional Biochemicals Corporation, Cleveland, Ohio, and were used in stock solutions of varying concentrations. These stock solutions were made up fresh each day and, unlike the one-half percent diastase of malt stock solutions, were not filtered.

The soluble starch (C. P., powder) was purchased from The Coleman and Bell Co., Norwood, Ohio. It was prepared in one percent concentration by adding one gm to 100 ml of boiling distilled water and stirring until completely dissolved.

Two methods were used to determine the rate and amount of diastase activity: (1) Munson-Walker values as described in Lange's Handbook of Chemistry (1939, page 1012), which involved a determination of the maltose produced as the result of the diastase activity; (2) a determination of the time required for starch to reach the colorless stage with I_2-KI (Eyster, 1953).

The pH was regulated by the use of MacIlvaine buffers, and the pH of all buffered digests was checked on the Beckman Glass Electrode pH Meter. In digests of 100 ml, 40 ml of buffer at each desired pH were used. In digests of 50 ml, 20 ml of buffer were used. At 25°C the digests usually had a total volume of 100 ml but occasionally, where specified, the total volume was 50 ml. At higher temperatures it was more convenient to use 50-ml digests. The tem-

peratures were maintained within one degree of the desired temperature by means of a water bath.

All Munson-Walker determinations were accompanied by blank runs which contained identical amounts of soluble starch but to which an identical amount of diastase inactivated by boiling (one min) had been added. Net milligrams of maltose values were obtained by subtracting the average for the blank runs from the average for the experimental runs.

TABLE 1

Effect of pH on diastase activity

pH	Diastase activity†	
	Without NaCl min	With 0.05 N NaCl min
2.2	∞	∞
3.0	∞	∞
4.0	∞	∞
4.85	270	135
5.98	35	28
6.98	35	28
7.98	85	40
9.0	∞	∞

†Diastase activity measurements were made with I_2KI reagent, and denote the time in minutes required for diastase to convert starch to the colorless stage.

These digests were run at 25° C, and contained 25 ml 1% soluble starch, 1 and ½ ml ½% diastase, 40 ml buffer, and 33 and ½ ml water.

DATA AND RESULTS

Using a series of MacIlvaine buffered solutions the effective pH range of diastase was found to be from four to nine with an optimum at from six to seven (tables 1 and 2, and fig. 1). It can be observed that the temperature of the digests did not seem to affect the optimum pH value. Munson-Walker values secured from digests at 25°C were quite similar to Munson-Walker values secured from digests at 40°C. Diastase activity measurements by the two methods gave good agreement as can be seen by comparing the data in table 1 with the data in table 2. In the former, diastase activity at different pH levels was determined by measuring the time required for a known amount of added soluble starch to reach the colorless stage with I_2-KI, and in the latter method the measurements involved a determination of the maltose produced as a result of diastase activity at various pH levels.

TABLE 2

Effect of pH on diastase activity

pH	Diastase activity 25° C 20 min	pH	Diastase activity 40° C 10 min
	mg maltose		mg maltose
5.23	57	5.0	43
6.13	118	5.81	96
6.99	109	6.71	106
7.97	64	7.68	42

Diastase activity measurements are Munson-Walker determinations and denote net mg of maltose formed. All measurements are averages of two determinations with the blanks averaging 9 mg maltose.

These digests contained 25 ml 1% soluble starch, 1 and ½ ml ½% diastase, 20 ml buffer and 3.5 ml distilled water.

In table 1 there is also a comparison of diastase activity at different pH levels with and without added chloride. Chloride ions were added as NaCl in a concentration of 0.05 M, the optimum for maximum stimulation of diastase activity at 25°C being 0.01 M (Eyster, 1953). The chloride anion produced a marked stimulation at all effective pH levels. However, the addition of chloride to diastase digests produced no change in either the enzyme's optimum pH or in its effective pH range.

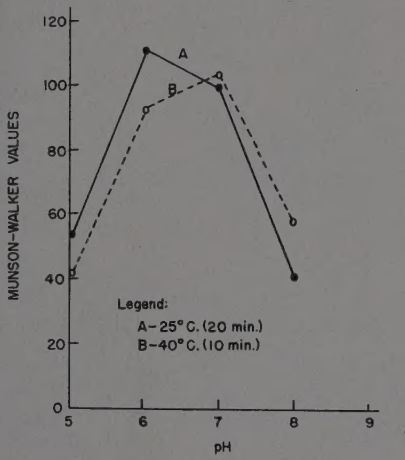

FIGURE 1. The effect of pH on diastase activity based on Munson-Walker determinations.

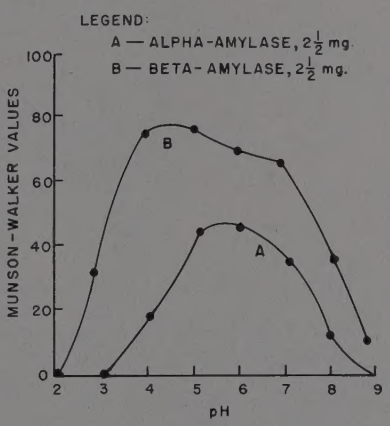

FIGURE 2. The effect of pH on alpha-amylase and beta-amylase activities based on Munson-Walker determinations of 15-min digests performed at 25° C.

Table 3 presents the effect of hydrogen ion concentration on β-amylase activity. These data are Munson-Walker determinations of the amount of maltose formed as a result of the action of β-amylase. The pH range was very broad extending from about pH 2 to about pH 9, and there was an optimum at about pH 5. The optimum hydrogen ion concentration was not very marked because there was considerable activity from as low as pH 4 to as high as pH 7.

The effect of hydrogen ion concentration on α-amylase activity is given in tables 4 and 5. In table 4 the measurements were made with I_2-KI reagent and denote the time in minutes required for α-amylase to convert starch to the colorless stage. In table 5 the amylase activity measurements were Munson-Walker determinations, and denote the net milligrams of maltose formed. The pH range was relatively narrow extending from about pH 4 to about pH 8. The greatest activity was from about pH 5 to about pH 7, being quite pronounced in table 4. The optimum pH was about six as indicated by the disappearance of starch, or about five and six as indicated by the amount of maltose formed. The pH activity range and the optimum pH for α-amylase were essentially the same as 40° C as at 25° C.

The activity rate of two and one-half mg β-amylase was found to exceed that of two and one-half mg α-amylase. Figure 2 presents the activity curves of 15-min digests performed at 25° C for both α-amylase and β-amylase over the entire effective pH range of each.

Table 6 shows what happened when α-amylase and β-amylase were mixed in a starch digest. Experiments were run at 25° C. As measured by the I_2-KI starch disappearance test, the pH range did not vary. The pH optimum was con-

TABLE 3

Effect of pH on β-amylase activity

pH	Amylase activity 25° C for 15 min
	mg maltose
2.15	0.2
2.9	26.7
4.0	74.3
5.0	77.6
6.0	70.9
6.9	67.6
8.0	37.5
8.8	12.5

Amylase activity measurements are Munson-Walker determinations, and denote net mg maltose formed. All measurements are averages of two determinations.

The digests contained 25 ml 1% soluble starch, 5 ml β-amylase from 50 mg β-amylase per 100 ml stock solution, and 20 ml buffer. Blanks with β-amylase boiled 1 min averaged 5.4 mg maltose.

sistently at pH 6, and amylase activity was always slightly greater at pH 5 than at pH 7.

Results of additional experiments conducted were as follows (data not given): (1) Diastase purified by the method given in Meyer and Anderson's plant physiology laboratory manual (1941, page 67) gave the same pH curve of activity as the original commercial material. (2) Clark and Lub buffers furnished the same pH activity curve as when MacIlvaine buffers were used. (3) The addition of chloride did not stimulate the activity of β-amylase. Zero, five, 25, 50, and 100 mg NaCl were added to successive digests of 50 ml each at 25° C. These measurements were made by the Munson-Walker method. The amount of chloride and other anions present in the β-amylase should have been determined as there may have been a sufficient quantity of anions in the commercial preparation to give maximum activity. (4) The activity of α-amylase was stimulated much less by the addition of chloride than was the activity of diastase. Chloride measurements revealed that each 0.5 gm of α-amylase contained chlorides equivalent to 25 mg NaCl as compared to 9.7 mg NaCl for each 0.5 gm of diastase of malt. Hence, it appears that the comparatively small chloride stimulation of the α-amylase

TABLE 4

Effect of pH on α-amylase activity

pH	Amylase activity 25° C min	pH	Amylase activity 24.5° C min
2.2	∞	2.2	∞
3.0	∞	3.0	∞
3.99	∞	3.95	∞
4.98	155	5.00	116
5.95	120	6.00	106
6.82	160	6.90	150
7.85	∞	7.9	∞
8.8	∞	8.8	∞

Amylase activity measurements were made with I_2KI reagent and denote the time in minutes required for α-amylase to convert starch to the colorless stage.

The digests contained 50 ml 1% soluble starch, 2 ml α-amylase solution from 50 mg α-amylase per 100 ml stock solution, 40 ml buffer, and 8 ml water.

TABLE 5

Effect of pH on α-amylase activity

pH*	Amylase activity		
	2½ mg	5 mg 40° C	25 mg 40° C
	mg maltose	mg maltose	mg maltose
2.15	0.8		
3.0	0.5		
4.05	17.5	40.9	52.6
5.15	42.6	67.4	86.2
6.12	43.4	66.6	85.7
7.0	35.1	61.0	78.5
8.0	11.7	39.8	52.6
8.9	−2.3		

Amylase activity measurements are Munson-Walker determinations and denote net mg maltose formed in 15-min digests. All measurements are averages of two determinations.

*pH of digests at 40° C (5 mg) were 4.15, 5.15, 6.1, 7.0, and 7.9. pH of digests at 40° C (25 mg) were 4.0, 5.1, 5.95, 7.05 and 8.0.

was due to its higher anion content. (5). The stimulation of diastase activity by nitrate and chloride anions was found to be additive. (6) The diastase accelerator in onion juice reported by Eyster (1948) was found to be due mostly to the chloride anion present. Since there were small amounts of additional anions present, it may be concluded that the total acceleration could be the result of the sum total of the stimulation produced by each of the anions present. (7) Variations in the optimum temperature for diastase of malt activity occurred with changes in the hydrogen ion concentration. At pH 8, the optimum temperature was approximately 50° C; at pH 6, approximately 40° C. These measurements were based on the rate of disappearance of starch using I_2-KI reagent. (8.) The optimum temperature for α-amylase activity was approximately 50° C at pH 5.95 as measured by the Munson-Walker method. (9) The optimum temperature for β-amylase activity ranged from 40 to 50° C at pH 5.0 for 5-min Munson-Walker digests. (10) Whereas 1 M glucose and 1 M sucrose retarded diastase activity about one-half (Eyster, 1942), only about 0.1 M maltose was required to produce a similar reduction in diastase activity. (11) Maltose retarded diastase activity as much at pH 7 as at pH 6.

DISCUSSION

Diastase is a mixture of enzymes which digests amylose (starch) to maltose. Diastase of malt consists of α-amylase, β-amylase, phosphatase, and numerous inorganic and other organic compounds. Beta-amylase is the enzyme which

TABLE 6

Digestion of soluble starch by mixtures of α- and β-amylases

pH	1 mg α	1 mg α + 1 mg β	1 mg α + 2 mg β	1 mg α + 3 mg β	1 mg α + 4 mg β	1 mg α + 8 mg β	2 mg α + 2 mg β
4.13	∞	∞	∞	∞	∞	∞	∞
4.95	116	98	79	72	75	75	31
6.0	106	80	68	63	60	60	23
7.15	150	113	107	105	105	103	37
8.1	∞	∞	∞	∞	∞	∞	∞

Amylase activity measurements were made with I_2KI reagent and denote the time in minutes required for the amylases to convert starch to the colorless stage.

The digests were run at 25° C and contained 50 ml 1% soluble starch, 40 ml buffer, and 10 ml of aqueous amylases and water.

converts amylose quantitatively to maltose and which is able to hydrolyze amylopectin to the residual dextrin. Dormant starchy seeds contain β-amylase, and as the seed germinates α-amylase appears. Alpha-amylase digests both amylose and amylopectin with the production of dextrins containing six or 12 glucose units, depending on whether there are one or two complete turns of the starch helix. The ability of starch to give a blue color with iodine is rapidly lost in the presence of α-amylase but is retained in the presence of β-amylase because of its inability to fully digest amylopectin. The general action of α-amylase is to produce progressively smaller and smaller particles from amylose and amylopectin, while β-amylase hydrolyzes amylose by successive removal of maltose units from the ends of the amylose chains inward. Beta-amylase also partially hydrolyzes amylopectin in a similar manner but the successive removal of maltose units from the ends stops at the first branch (Bonner, 1950). Hanes (1937) has shown that dextrins do not give an iodine color reaction if they contain six or fewer glucose units, that dextrins containing eight or 12 glucose units form a red complex, and that only the longer amylose chains give the typical blue iodine color. Phosphatase is the enzyme which liberates phosphate from phosphorylated sugars.

There is little or no diastase in chloroplasts, and the sugar to starch and starch to sugar reactions in chloroplasts are over pathways facilitated by phosphorylase. The study of diastase, however, has proven especially interesting and worthwhile because of the chloride ion and because of anions in general which accelerate not only diastase activity but also Hill reactions (Arnon and Whatley, 1949) and growth rates of *Chlorella pyrenoidosa* (Eyster, 1958).

SUMMARY

The effective pH range of diastase as determined by two different methods was found to be from four to nine with an optimum at from six to seven. Both the pH range and the pH optimum remained unchanged in digests at 40° C as compared with digests at 25° C. Although chloride anions markedly accelerated diastase activity, they altered neither the pH range nor the pH optimum.

The pH range for β-amylase extended from about pH 2 to about pH 9. Considerable activity occurred between pH 4 and pH 7, and a weak optimum appeared around pH 5. Alpha-amylase showed a pH range of about pH 4 to pH 8. Its greatest activity was exhibited from pH 5 to pH 7, and its optimum was around pH 5 and pH 6. Using equal amounts of α-amylase and β-amylase in separate digests, it was found that the activity rate of β-amylase exceeded that of α-amylase.

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THE ACCUMULATION AND DISTRIBUTION OF P^{32} IN VARIOUS TISSUES OF NITROGEN-, POTASSIUM-, CALCIUM-, AND MAGNESIUM-DEFICIENT CORN PLANTS[1]

TZU-LIANG YUAN[2] AND J. D. SAYRE[3]

The absorption of mineral nutrient elements by a plant may be affected by a number of factors, including the synergisms and antagonisms of different elements. The effect of such factors on the distribution and accumulation of the mineral elements in various plant tissues has not been thoroughly investigated. In the experiments reported here P^{32} was used to measure the phosphorus accumulation and distribution in corn plants that had been deficient in nitrate-nitrogen, ammonium-nitrogen, potassium, calcium, and magnesium.

Experimental Method

The corn hybrid, Ohio W64, was grown in the greenhouse, in 3-gallon pots containing quartz gravel, and was irrigated periodically with a mineral nutrient-solution by a compressed air system (Sayre, 1952). Four seeds were planted in each pot and the pots received only rain water for the first two weeks. Then the seedlings were thinned to two per pot and all pots received an optimum nutrient solution for three weeks, after which the solutions were changed to those shown in table 1. All treatments were replicated four times. After two weeks, these solutions were renewed except that P^{32}-tagged phosphate replaced ordinary phosphate. The plants were harvested two weeks later, after a total of nine weeks of growth and four weeks of deficiency treatment.

The plants were dissected into tassel, buds, leaves, sheaths, nodes, and internodes. All corresponding tissues (counting from the one just below the tassel) from replicated plants were composited and dried in a 70°C oven for 72 hours. Then samples were ground with a portable mill and passed through a 30-mesh sieve. Duplicated samples were analyzed in a Geiger counter for the radioactivity of P^{32}. All data were corrected with the conventional factors (Yuan, 1952) and computed to the radioactivity of P^{32} in the corn tissues at the time the plants were harvested.

Results and Discussion

Plants in optimum solution.—The accumulation of P^{32} in various tissues of the different positions on the plant is shown in figure 1. Phosphorus ^{32} accumulated in the leaves and sheaths was at a lower but more uniform concentration than in the other tissues. The node, followed by the internodes, had the highest concentrations in the top of the plant. Both fell off sharply in the basal part of the plant.

Since the phosphorus is utilized largely in young, meristemic cells of the growing regions for the formation of nucleoproteins and other phosphorus-containing compounds, it would seem logical for the lower, older leaves, sheaths, nodes and internodes to contain less phosphorus. Figure 1 shows only a small downward gradient for that phosphorus in the lower leaves. The phosphorus in

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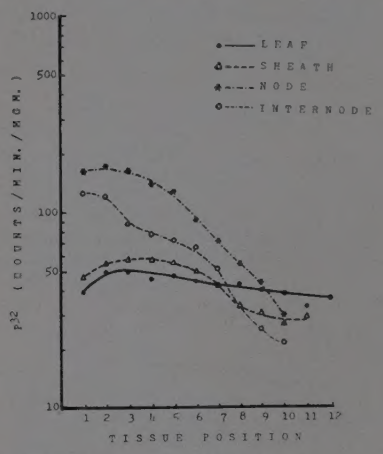

FIGURE 1. Accumulation of P^{32} in the individual tissues of normal corn plant.

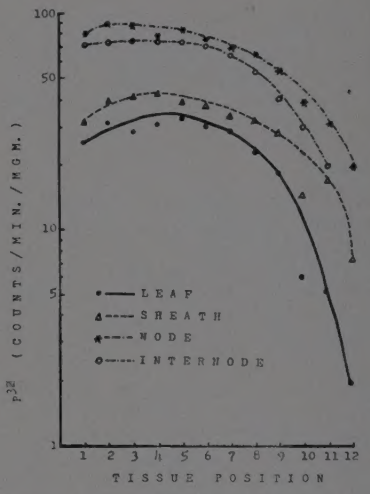

FIGURE 2. Accumulation of P^{32} in the individual tissues of nitrate-nitrogen deficient corn plant.

the sheaths showed a gradient that was greater than in the leaves, but less than in the nodes and internodes at different positions on the plant.

Nitrate-nitrogen deficient plants.—The accumulation of P^{32} in the tissues of nitrate-nitrogen deficient plants is shown in table 2 and figure 2. The P^{32} content of all tissues except the nodes was much lower than that in the other treatments. The P^{32} concentration in different tissues of corresponding positions decreased in the order of nodes, internodes, sheaths and leaves without exception and fell off very sharply in the older leaves and sheaths as well as the nodes and internodes. The gradient was greater than in any of the other treatments.

TABLE 1

*Concentration (ppm) of various elements in the nutrient solution of different treatments**

Element	Treatment					
	Check (opt.)	NO_3–N deficient	NH_4–N deficient	K deficient	Ca deficient	Mg deficient
Na	29	29	37	89	144	66
K	100	100	100	0	100	100
Ca	100	100	100	100	0	100
Mg	20	20	20	20	20	0
NH_4–N	5	5	0	5	5	5
NO_3–N	95	0	95	95	95	95
P	10	10	10	10	10	10
S	52	52	52	52	52	52
Cl	16	253	16	16	16	16
Total**						
cations	254	254	257	214	269	271
anions	173	315	173	173	173	173

*All solutions also received a half unit of minor elements mixture used by Sayre (1952).
**Ions from micronutrients are not included.

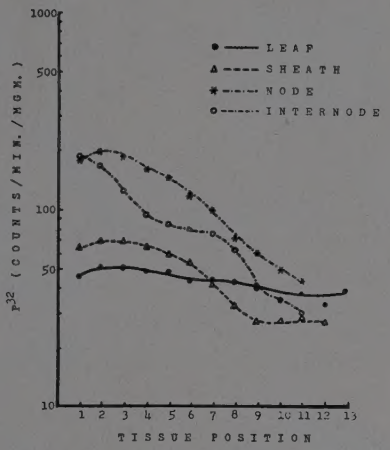

FIGURE 3. Accumulation of P^{32} in the individual tissues of ammonium-nitrogen deficient corn plant.

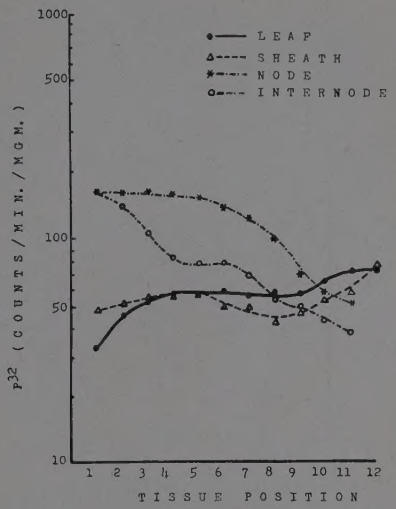

FIGURE 4. Accumulation of P^{32} in the individual tissues of potassium-deficient corn plant.

The low uptake of P^{32} found in the plant tissues from this treatment is believed to have been caused by the deficiency of nitrate-nitrogen, or total nitrogen, or to an excess of chlorides. Leonard et al. (1948), in their studies on sweet potatoes, concluded that nitrate and phosphate ions mutually benefit each other with respect to absorption but no adverse effect was observed. In our experiment, in order to maintain the same concentration of calcium in the nitrate-nitrogen deficient mineral nutrient solution, calcium nitrate was replaced with calcium chloride. Therefore, the concentration of chloride ions was greatly increased (see table 1). Since the chloride ions may contribute a large share to total anion content of the plant and may be involved in ionic competitive effects indicated by Wallace et al. (1949), the high concentration of chloride ions in the mineral nutrient solution may also suppress the phosphorus absorption.

The phosophrus uptake was low under the nitrate-nitrogen deficient treatment and the plants suffered a phosphorus deficiency despite its ample supply in the solution. The extremely low P^{32} concentration in the older leaves may indicate

TABLE 2

*Concentration of P^{32} accumulated in different tissues**

Plant Tissue	Treatment					
	Check (opt.)	NO_3—N deficient	NH_4—N deficient	K deficient	Ca deficient	Mg deficient
Tassel	52.7	43.2	63.2	97.2	57.6	63.7
Buds	115.8	75.4	126.3	182.0	114.5	107.4
Leaves	44.4	27.0	45.7	58.0	38.9	52.4
Sheaths	46.7	34.5	46.2	51.6	42.5	49.1
Nodes	78.4	62.2	87.7	103.8	74.6	79.4
Internodes	55.3	60.4	60.9	66.0	57.6	66.6

*Counts per minute per milligram tissue.

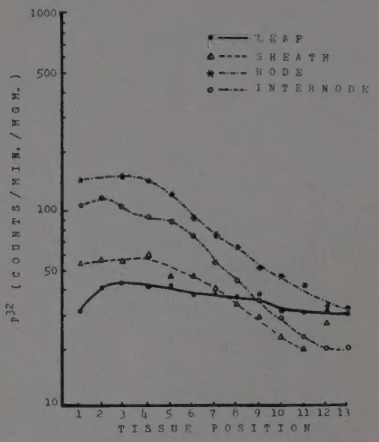

FIGURE 5. Accumulation of P^{32} in the individual tissues of calcium-deficient corn plant.

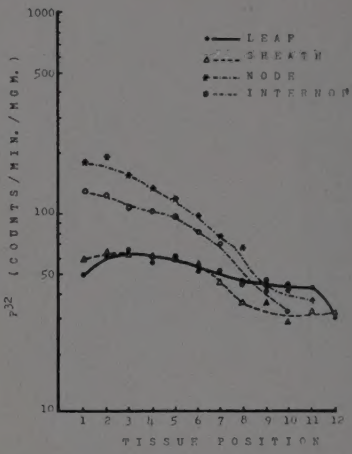

FIGURE 6. Accumulation of P^{32} in the individual tissues of magnesium-deficient corn plant.

that these leaves were no longer functioning and that an upward redistribution of phosophorus occurred. The young growing tissues gained in phosphorus content at the expense of the older ones.

Ammonium-nitrogen deficient plants.—Figure 3 shows the accumulation of P^{32} in various tissues of the corn plant in which ammonium-nitrogen deficiency was supposed to occur. The curves are very similar to those obtained from the check plants except that the phosophrus concentrations in nodes, internodes and the upper sheaths were higher than in the corresponding tissues of the check plants. It seems that ammonium-nitrogen deficiency had little effect on the accumulation of phosphorus, probably because the ammonium-nitrogen comprised only five percent of the total nitrogen in the nutrient solution.

Potassium deficient plants.—The differential accumulation of P^{32} in the different tissues of the plants grown in the solution in which potassium was deficient is shown in figure 4. The omission of potassium from the solution resulted in an increased accumulation of P^{32} in the older leaves and sheaths, a condition opposite to that occurring when a complete nutrient solution was used.

The increase of phosphorus accumulation in plant tissue due to potassium deficiency has been observed previously. Mulder (1952) studied the nutritional interrelationship of magnesium, potassium, and phosphorus in apple leaves and found that samples with a low potassium content contained larger amounts of phosphorus than did normal ones. The results of our experiment show that the same relationship may occur in the different parts of the same plant. Phosphorus was building up in the older leaves and sheaths which are known to lose their potassium to the younger tissues under early stress of potassium deficiency.

Calcium deficient plants.—Figure 5 shows that the accumulation of phosphorus in the individual tissues of calcium deficient plants was very similar to that occurring in normal plants, as shown in figure 1. The lower nodes had a higher concentration of phsophorus than did those in normal plants. The deficiency of calcium might cause a stunting of the root system and reduce the absorption of total phosphorus by the plant.

Magnesium deficient plants.—Phosphorus accumulation in various tissues of

magnesium deficient plants, as shown in figure 6, was the same as that for normal plants, except for minor differences between the curves for leaves and internodes.

Accumulation and distribution of P^{32} in different tissues.—Table 2 shows the unit concentration of P^{32} in different tissues of the corn plant. The highest concentration is found in the buds (ear), twice as much as in any other tissues except the nodes which had about two-thirds the concentration of the buds. Internodes and tassel came next. Sheaths and leaves had the lowest concentrations. The deficiency treatments influenced the P^{32} concentration mostly in the tassel, buds, leaves and nodes.

All deficient treatments reduced the percentage distribution of P^{32} in the tassel, buds, and sheaths except for the plants deficient in potassium which had a slightly higher percentage of P^{32} distributed in the tassel (table 3). The nitrate-nitrogen deficiency increased the P^{32} percentage in nodes and internodes. Other treatments apparently had no effect. The greatest influence of deficiency treatments seemed to be on leaves. Nitrate-nitrogen deficiency decreased the percentage content of phosphorus, but the percentage accumulation of P^{32} increased in leaves of all corn plants of other treatments. This latter observation is in accordance with those reported by Evans et al. (1950).

TABLE 3

Percentage distribution of total P^{32} in various tissues of the aerial part of the corn plant

Plant Tissue	Treatment					
	Check (opt.)	NO_3—N deficient	NH_4—N deficient	K deficient	Ca deficient	Mg deficient
	%	%	%	%	%	%
Tassel	13.0	11.4	10.9	14.0	12.5	10.5
Buds	3.2	0.5	1.4	1.1	1.0	1.6
Leaves	38.5	29.9	44.2	47.3	42.6	45.6
Sheaths	17.2	16.7	14.5	14.8	16.1	15.6
Nodes	7.3	10.2	7.4	7.0	7.4	6.3
Internodes	20.8	31.2	21.6	15.8	20.4	20.4

Summary

The accumulation and distribution of P^{32} in various corn tissues as influenced by the deficiencies of various major nutrient elements were studied. The highest concentration of P^{32} was found in the upper leaves, sheaths, nodes, and internodes with a gradual decrease down the stalk. The gradient was less in the leaves and sheaths than in the nodes and internodes in most of the treatments, except that involving nitrate-nitrogen deficiency. High concentrations of P^{32} were observed in the lower leaf and sheath tissues of the potassium deficient plants.

About 40 percent of the total P^{32} in the aerial part of the plant accumulated in leaves, 20 percent in internodes, 17 percent in sheaths, 13 percent in tassel, and seven percent and three percent each in nodes and buds of the check or normal plants. This distribution was somewhat different in plants growing in deficient nutrient solutions. Nitrate-nitrogen deficiency decreased the total P^{32} absorption and the proportion in the leaves, increased the proportion of P^{32} in the nodes, and internodes, with no change in other tissues. Ammonium-nitrogen, calcium, and magnesium deficiency treatments increased the accumulation in the leaves and decreased it in tassel, buds and sheaths while in nodes and internodes, the percentage was constant. A deficiency of potassium resulted in plants with a much

higher percentage of P^{32} in leaves, higher in tassel but lower in buds, sheaths, and internodes as compared with the normal plants while the percentage of phosphorus in the nodes was constant.

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OBSERVATIONS ON THE CLOACAL GLAND OF THE EURASIAN QUAIL, *COTURNIX COTURNIX*

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While handling the Eurasian quail, *Coturnix coturnix*, in a game stocking program, the junior author noted that the breeding males issued a frothy fluid from their cloacae. Upon examination we noted that this secretion originated in a gland located above the dorsal lip of the cloaca. There is a hypertrophy of this glandular tissue which is associated with enlarged testes (15 x 25 mm in size) and with great behavioral sexual activity of the male. There seems to be little doubt that this secretion is associated with the mechanics of internal fertilization. There exudes a copious, clear secretion from many small pores over the surface of the gland. The secretion becomes full of air bubbles and fills the cloaca with a meringuelike froth. It is also evacuated on defecation and clings to the droppings; the latter occurrence causes caged birds to accumulate large balls of dung on their toes. The froth is transferred to the cloaca of the female during copulation.

Tissues were removed from freshly-killed birds and placed in San Falice's, Carnoy's, and corrosive acetic fixatives. Sections were cut 12 to 14 μ thick and were placed on slides (cleaned with acid) without the use of albumin. The stains used were periodic acid Schiff, Harris' haematoxylin, azure B (buffered at 4.0) and periodic acid-celestin blue (Pearse, 1950). Acetone, ethyl alcohol and xylene dried with silica gel were used for dehydration.

In this bird, the dorsal lip of the cloaca extends ventrad (about five mm) outside the ventral lip which is internal to the other lip. The dorsal lip is thin and only slightly muscular with a thin layer of circular and longitudinal muscles.

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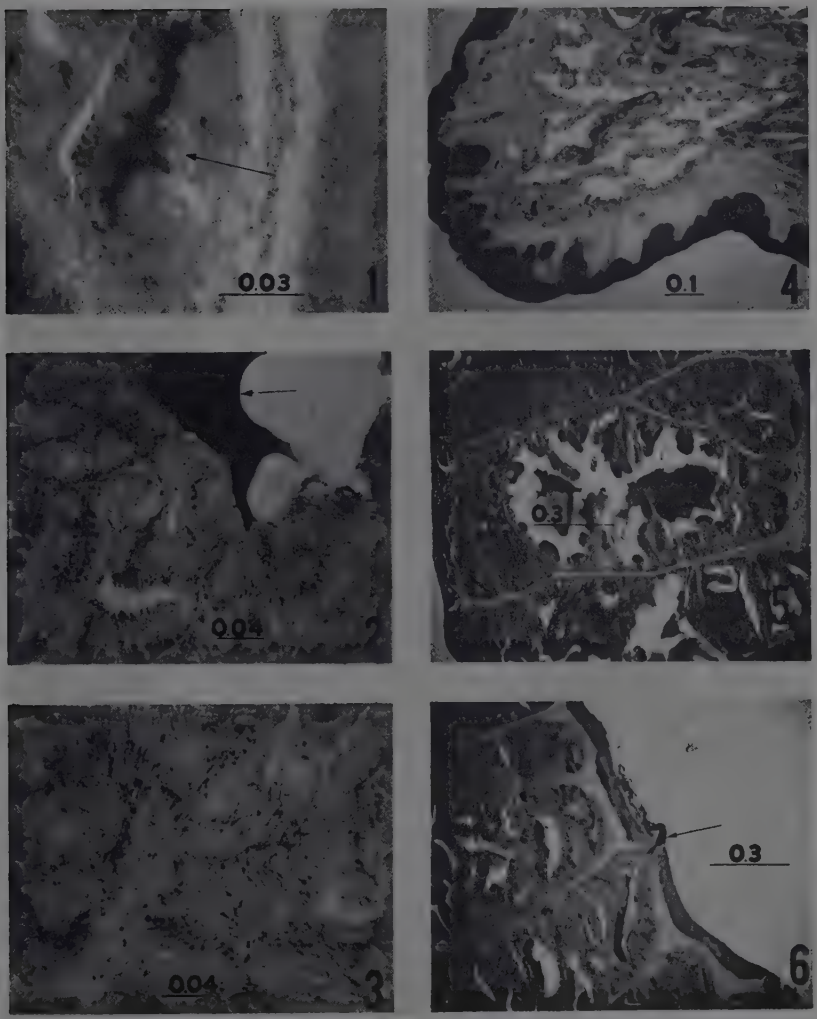

EXPLANATION OF FIGURES IN PLATE

All tissues shown in this plate, except figure 1, were stained periodic acid Schiff-celestin blue. All measurements are in millimeters.

1. A section stained with periodic acid Schiff showing the positive reaction of the secretion adjacent to the epithelium.
2. A collection of the secretion in the lumen of the gland.
3. A section showing the granular nature of the secretory cytoplasm.
4. A section through the penis.
5. A sagittal section showing the arrangement of the sections.
6. A sagittal section showing a pore and its secretion.

The lining of the lip is heavy, stratified epithelium and this extends to the juncture with the proctodeum. The ventral lip is heavy, short and extends up inside the long arc formed by the dorsal lip. The low columnar epithelium characteristic of the proctodeum (Calhoun, 1933) is present at the base of the ventral lip. Near the border of this lip, there is a large circular muscle.

The gland studied here is embedded in the dorsal lip of the cloaca and extends from the heavy musculature at the base of the bursa of Fabricus almost to the tip of the dorsal lip. The size of the gland is about ten mm long (measured around a curve) and two mm thick. In the breeding male (sexual maturity about seven weeks) the crissum is greatly distended, accommodating the enlarged gland, and it is colored a dark red externally. The swelling is useful in determining the sex of live birds in questionable plumage. This swelling is not to be confused with that of passerine birds (Wolfson, 1954) which contains convoluted sperm ducts. The vas deferens and ureter open independantly of the cloacal gland in the quail.

The gland is divided by connective tissue septa forming units which are longer than broad (fig. 5). Each of these segments opens to the outside by means of a small pore (fig. 6). Frequently these pores are reenforced by stratified epithelium thickenings and other times the pore appears only as a hole through the epithelium (which could be confused with a simple break in the tissue). The individual unit of the gland is tubular in shape (fig. 1). These are arranged so that the effect is an alveolar gland. The ducts are not readily apparent and they are lined with a low columnar epithelium. The typical glandular epithelium is a high columnar type with a nucleus in the basal third of the cell. The cytoplasm contains a network with small granules.

We have good evidence to believe that this secretion is mucoid in nature. It stains readily with the periodic acid Schiff reagent and clearly violet with toluidine blue (the latter is a specific test for mucin). The cytoplasmic granules are positive for these two stains also (fig. 1 and 2). Both the secretion and the granules are resistant to digestion by malt diastase and ribonuclease. The secretion removed from the bird, smeared on a slide, and air dried, gives the same reactions as the secretion found in the ducts in the sectioned material.

In the male bird there is a penislike structure on the ventral wall of the cloaca (fig. 4). The structure is quite small, but it can be located readily by the pigment present in it. The penis appears to be erectile; sinuses lined with epithelium are present in it. We did not observe the penis in the turgid state and since we do not know the size of it in the distended state, we can only speculate concerning its relationship to the gland. If the penis is inserted during copulation, then it is possible that the secretion of the gland may serve as lubrication. Although the presence of a penis is well-known in water fowl, it has not been reported in gallinaceous birds.

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CONCENTRATION OF TOBACCO MOSAIC VIRUS IN TOMATO PLANTS THROUGHOUT THE GROWING SEASON OF TWO GLASSHOUSE CROPS AND A FIELD CROP[1]

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INTRODUCTION

Commercial glasshouse tomato crops generally become 100 percent infected with green strains of the tobacco mosaic virus. Infection frequently becomes prevalent shortly after the plants are set in the soil. Field grown tomatoes are less universally infected, but where early infection occurs the losses are considerable.

Alexander (1949, 1950, 1951), Heuberger and Moyer (1931), Heuberger and Norton (1933), Jones and Burnett (1935), and Selman (1942) have studied the effect of tobacco mosaic virus on the yield of tomatoes; and Alexander (1949), Heuber and Moyer (1931), and Heuberger and Norton (1933) have shown that the reductions of yield of early infected field tomatoes may be as high as 50 percent. These workers point out that decrease in yield is greatest when plants are infected early. Moreover, Alexander (1949, 1950, 1951), Jones and Burnett (1935), and Selman (1942) have shown that the reduction in yield of glasshouse tomatoes due to mosaic varies with the season, the decrease in yield appearing to vary from 10 to 25 percent. Selman (1942) first pointed out that fruit set was reduced at the time of infection. Alexander (1950, 1951) further emphasized this fact and, by a study of the average fruit set for each cluster throughout a glasshouse tomato crop, showed that tomato plants infected early with mosaic set few fruits on the early clusters and then made a remarkable recovery. In fact, the fruit set on later clusters was slightly above average.

A decrease in the virus content of the plants might be an explanation for the partial recovery. Accordingly, it was decided to follow the active virus concentration of infected plants throughout one or more growing seasons in order to determine whether there was a drop in the active virus content of the plants which correspond to the recovery of the plants.

METHODS AND MATERIALS

Experiments designed to study the effect of tobacco mosaic infection on tomatoes at different stages of growth are described in detail elsewhere (Alexander, 1949, 1950, 1951). Inasmuch as the same plants were used in this study, a brief description is repeated here. The tomato plants were grown in a glasshouse which was divided twice both lengthwise and crosswise, making a total of nine plots. The plants in three plots were inoculated January 18, and the plants in another three plots on March 28. The plants in the third set of plots were uninoculated and were intended to be kept healthy as controls. However, they accidentally become contaminated about the first of May. In 1950 one-half of each plot was planted with plants of the variety Ohio W-R Globe and one-half with plants of the variety Strain A Globe. Thirteen plants of each variety were used. In 1951, only the variety Ohio W-R Globe was used.

In 1951, the virus concentration of inoculated, field grown, Stokesdale plants was followed. The plants were unpruned and unstaked. Ten plants were used for each plot and the plots were replicated four times. The plants in one set of

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plots were inoculated June 15, the plants in another set on July 16, and the plants in a third set were left as healthy controls.

In all cases, the plants were inoculated by dusting three or more leaflets with carborundum and then rubbing them with juice from infected plants. In the glasshouse work, inoculum was prepared from a diseased plant that appeared to be infected with a normal green strain of the tobacco mosaic virus. In the case of the field experiments the initial inoculation was prepared in 1950 from a plant that appeared to be infected with a normal green strain of the virus, and in 1951 the initial inoculum was a purified virus suspension prepared from tomato plants that appeared to be infected by a normal green strain.

The virus concentration was estimated by the local lesion method described by Holmes (1929a, b) and Samuel and Bald (1933) using *Nicotiana glutinosa* as the assay plant. In the preliminary work of 1950 whole leaves were used, and five leaves on each of two plants were inoculated for each assay. In 1951, in order to insure a more even distribution of the 600 grit carborundum, a small Hudson hand duster was used. It was modified by replacing the quart dust container with a pint jar and shortening the discharge tube to approximately four inches. With this duster it was possible to get what appeared to be an even distribution of the carborundum on the leaves. The assay inoculum was rubbed over five half leaves on each of two plants.

Leaf samples for assaying were first collected from the tops of the tomato plants approximately two weeks after inoculation. Thereafter, samples were collected every two weeks. It seemed desirable to follow the virus content of the first leaves sampled; therefore, lower leaves were assayed as well as the top leaves. Somewhat later, as the plants approached maturity, the leaves in the middle of the plants were also assayed.

In the preliminary work of 1950, the assay inoculum was prepared by grinding 0.6 g of tomato leaf in 6.0 ml of water. In the more exact experiments of 1951, the assay inoculum was prepared from ten cm^2 of tomato leaf tissue. A leaf punch of one cm^2 area was used to obtain a sample from ten different plants. The ten cm^2 of leaf tissue were thoroughly ground with nine ml of water. This solution was then diluted 1:100, making an approximate dilution of the inoculum of 1:1,000. One half of each of five *Nicotiana glutinosa* leaves was inoculated with this inoculum. This dilution of the inoculum gave well distributed lesions.

The other half of the five leaves of each *Nicotiana glutinosa* plant was inoculated with a purified green strain of the tobacco mosaic virus complex. Precipitation of the virus complex was carried out at the isoelectric point, pH 3.4, following the work of Best (1936, 1948). Tissue macerated in a ball mill was diluted one to five with water and centrifuged at 1000 x *g* for about 20 min and then recentrifuged at 1500 x *g* for about two hr. The precipitate, consisting of plant debris, was discarded and the supernatant liquid was diluted with an equal volume of standard buffer, pH 3.4, giving a dilution of 1:10. The addition of the 3.4 pH buffer to the clarified juice gave a pH of approximately 4.0. Final adjustment to pH 3.4 was made with 0.1 N hydrochloric acid. The clarified juice was again centrifuged at 2000 x *g* for 30 min. The precipitated virus complex was diluted 1:10 with standard pH 7.0 buffer and was highly infective but was not infective at pH 3.4. This is in accord with the recent work of Takahashi (1949). The virus complex was reprecipitated, using 5.0 M glacial acetic acid to adjust to the isoelectric point. However, since it required undue amounts of acetic acid to attain a pH below 4.0, the final adjustment to pH 3.4 was made with 1.0 N hydrochloric acid. The precipitated virus complex was diluted to the original juice volume with a standard pH 4.0 buffer and stored in a refrigerator. When used, the virus suspension was diluted 1:1,000 with pH 7.0 buffer. Comparisons between the purified virus complex diluted 1:1,000 and freshly ground virus infected tomato leaves diluted 1:1,000 gave very similar numbers of local lesions on *Nicotiana glutinosa*.

In the preliminary work of 1950, the data are expressed as the number of lesions per square inch of leaf surface. However, in the work of 1951, the data are presented as the number of lesions per square centimeter of leaf surface of *Nicotiana glutinosa*. The area of the *N. glutinosa* leaves was obtained by measuring the breadth of the leaves at their widest point and the length of the leaves from the tip to the base of the blade. The product of these measurements does not give the exact area of the leaves, but a series of measurements by use of a planimeter on 49 leaves indicated that the actual area was approximately 86 percent of the

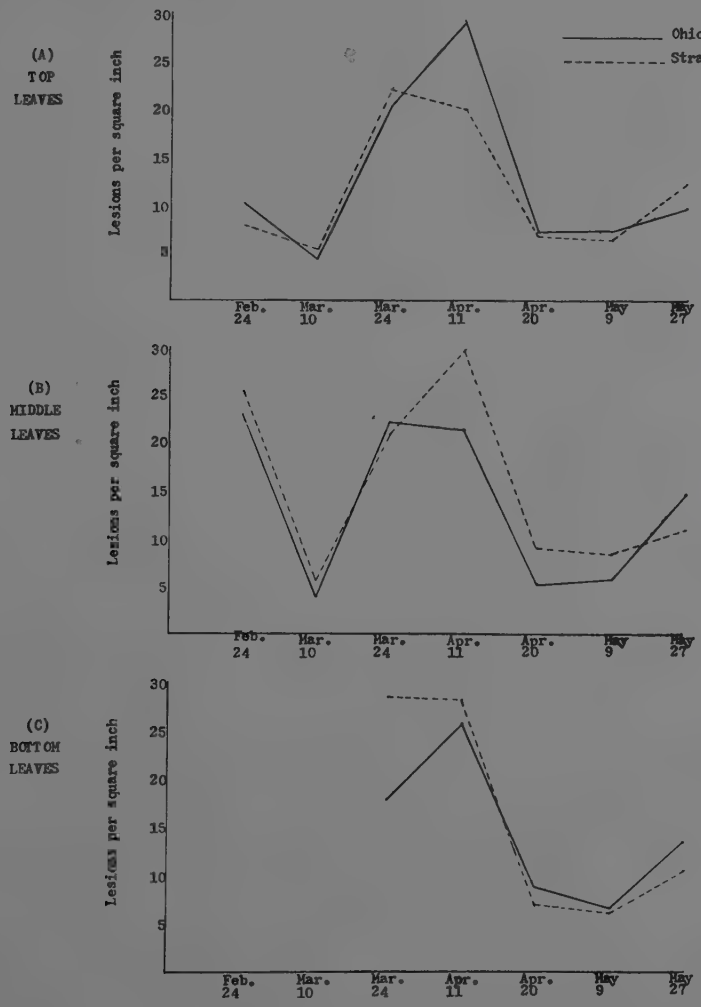

FIGURE 1. Tobacco mosaic virus concentration in tomato leaves expressed as average number of lesions per square inch for two tomato varieties. Inoculated January 18, 1950. Sam Dean's Glasshouse.

calculated area. However, since the correction would be a constant and the data are relative, the area was assumed to be the product of the width by the length.

RESULTS

Several methods of evaluating the effects of tobacco mosaic diseases on tomatoes have been used. In this investigation the number of fruit set per cluster is used as an indication of the effects of the disease. In order to accomplish this, a record of the number of fruits which were set per cluster was made for the entire growing season. This fruit-set record covers the same period as the virus assay.

During the analysis of the plants in 1950 the number of lesions per square inch which occurred on leaves of *Nicotiana glutinosa* was used as the index of virus content of the plants. However, in 1951 in order to refine the technique, the number of lesions per square centimeter on half leaves was expressed in terms of the number of lesions on the other half leaves inoculated with a purified virus preparation. This was done in order to eliminate, at least in part, the variability of different lots of assay plants.

TABLE 1

Influence of the tobacco mosaic disease on the number of fruits set per cluster for two greenhouse tomato varieties. Sam Dean's Glasshouse—Spring, 1950.

Treatment		Cluster number											Average fruits per cluster
		1	2	3	4	5	6	7	8	9	10	11	
						Strain A Globe							
Inoculated	1/18/50	1.9	4.0	4.6	5.8	5.0	4.7	5.0	4.6	3.6	3.9	3.4	4.2
Inoculated	3/28/50	3.9	4.8	5.2	5.1	4.3	4.2	4.3	4.6	3.9	4.1	3.8	4.4
Healthy		4.3	4.8	5.1	5.9	4.2	4.7	5.0	4.3	3.4	3.7	3.0	4.4
						Ohio W-R Globe							
Inoculated	1/18/50	2.2	4.5	4.7	5.2	4.7	4.9	5.4	4.1	3.7	3.6	3.6	4.2
Inoculated	3/28/50	4.1	4.8	4.8	5.0	4.3	4.2	5.2	5.4	4.1	4.0	4.0	4.5
Healthy		3.8	5.4	4.9	5.4	4.8	5.2	5.3	4.5	3.6	3.9	4.1	4.6

Fruit set record for 1950 and 1951.—In previous work Alexander (1950, 1951) showed that as glasshouse-grown tomato plants made a visible recovery following mosaic infection, the setting of fruit tended to approach normal or in some instances to exceed that of healthy plants. The fruit set data for the varieties, Ohio W R Globe and Strain A Globe, grown in the spring of 1950 are shown in table 1.

The number of fruits set on the early clusters of the Ohio W R Globe plants, inoculated, the middle of January, at the time of transplanting them into the permanent ground beds, was reduced (tables 1 and 2). Fruit set was improved on the second and third clusters and reached what appeared to be normal in the fourth and succeeding clusters. Similarly, fruit set on the variety, Strain A Globe, was reduced on the first clusters following inoculation (table 1). It is also shown (tables 1 and 2), for the variety Ohio W R Globe and table 1 for the variety Strain A Globe, that a marked reduction in fruit set occurred following the second inoculation made approximately ten weeks after the first. This reduction in fruit set occurred on the fifth, sixth, and seventh clusters for both varieties in the spring of 1950. In the spring of 1951, variety Ohio W R Globe, the sixth and seventh clusters were severely affected following inoculations. In the later clusters there was a general decline in fruit set on the plants of all plots. This general decline in fruit set may possibly be explained by the high temperatures of May and June.

Virus concentration, Glasshouse, 1950.—The active virus concentration in the two varieties, Ohio W-R Globe and Strain A Globe, was similar throughout the season (fig. 1 and 2). The concentration of tobacco mosaic virus in the plants inoculated January 18, 1950, appeared to fluctuate throughout the season. The data are shown in figure 1. The number of lesions per square inch of leaf of assay plants produced by inoculum secured from the top leaves for the first two sample periods, February 24 and March 10, varied between five and ten. The virus

TABLE 2

Influence of tobacco mosaic virus on the number of fruits per cluster, Ohio W-R Globe. Sam Dean's Glasshouse—Spring, 1951.

Treatment	Cluster number											Average fruits per cluster
	1	2	3	4	5	6	7	8	9	10	11	
Inoculated January 17, 1951.	1.58	2.77	3.46	5.82	4.41	4.34	4.99	4.89	3.93	3.63	2.45	3.84
Inoculated March 28, 1951.	4.53	5.44	4.60	4.44	4.17	2.69	1.93	3.58	3.61	3.82	3.33	3.83
Healthy	3.90	5.30	4.74	4.59	5.35	4.71	4.67	4.42	4.07	3.36	2.90	4.36

content of all leaves sampled during the next two sample periods, March 24 and April 11, increased markedly. Later in the season, the virus content again decreased. However, in this case, increase in virus content occurred at about the time the plants were exhibiting symptoms of recovery and fruit set was returning to normal. The concentration of the virus throughout the plant in the March 24 and later sample periods, as represented by samples from the top, middle, and bottom leaves, appeared to be about the same.

The concentration of the virus in the plants inoculated March 28, 1950, fluctuated somewhat but remained at a lower level than that attained in the first inoculation (fig. 2). The effect of this inoculation reduced fruit set (table 1). The plants inoculated March 28, 1950, were approximately five ft tall, and in this case the virus concentration, though present in the lower leaves, did not increase greatly. The concentration of the virus in the center portion of the plants approached that in the tops of the plants. This might be expected because the leaves in the center portion of the plant were immature at the time of inoculation.

Virus concentration, Glasshouse, 1951.—The virus content of Ohio W–R Globe plants was followed throughout the growing season of 1951. *Nicotiana glutinosa* was again used as the assay plant. However, the data are expressed as lesions per square centimeter of half leaf surfaces after correction according to the number of lesions produced by the purified virus on the adjoining half leaves.

The data are shown graphically in figure 3. As in 1950, the virus concentration in the tops of the plants inoculated January 17 was approximately the same as in the bottoms of the plants (fig. 3a). Again there did not appear to be any relationship between the virus concentration and the recovery by the plant, judging from its ability to set a normal number of fruits per cluster.

The virus concentration of the plants infected March 28 was much less uniform (fig. 3b). The results differed in that the virus concentration in the lower leaves remained low and then increased rapidly.

Virus concentration, Field tomatoes, 1951.—Using the same methods, the virus content of plants of the variety Stokesdale, field grown and unstaked, was followed for a growing season. The plants were transplanted to the field May 31.

The first inoculation was made on June 15 and the second on July 16. Ten plants were used per plot with three replications. The data are shown graphically in figure 4. Inadvertently, assays were not made for the first two periods on the tops of the plants inoculated June 15. The virus concentration increased in the bottom leaves until July 30, then it declined. The virus content in the top leaves of the plants infected July 16 was high, whereas the virus content of the bottom leaves was low initially but gradually increased.

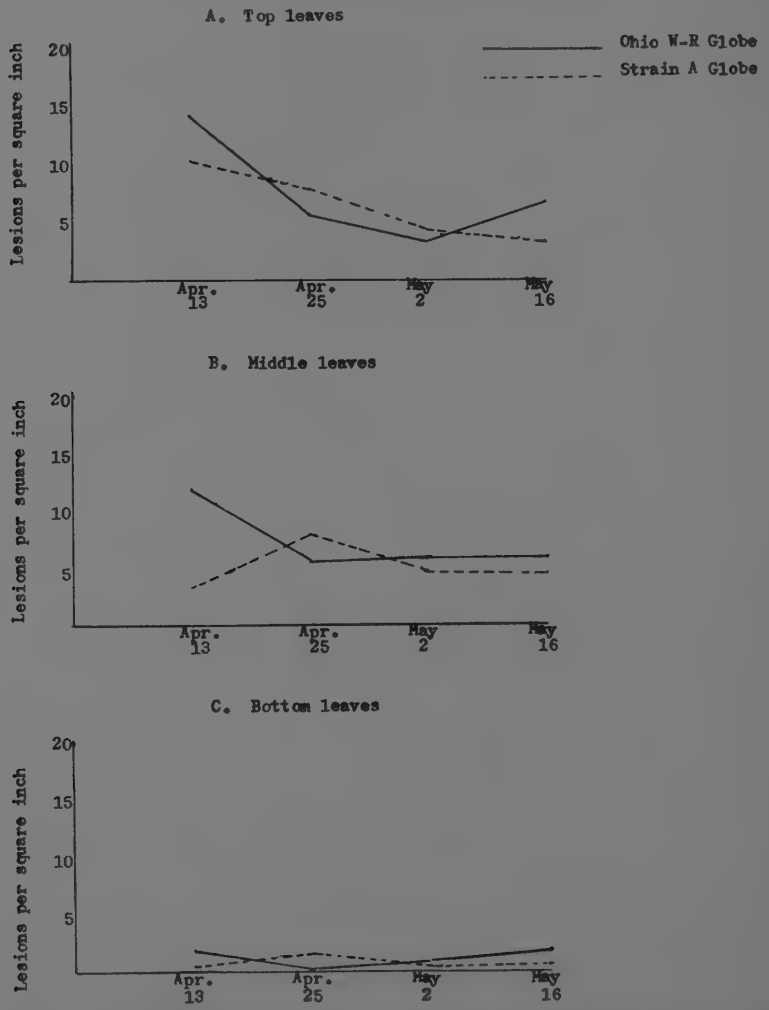

FIGURE 2. Tobacco mosaic virus concentration in tomato leaves expressed as average number of lesions per square inch for two tomato varieties. Inoculated March 28, 1950. Sam Dean's Glasshouse.

These data, however, do not indicate a relationship between virus content and recovery of the ability to set fruit. Fruit set data are not available but yield data previously published (Alexander, 1949, 1950, 1951; Heuberger and Moyer, 1931; Heuberger and Norton, 1933) have all shown that early infections result in the greatest loss. Alexander (1949 and unpublished data) secured yield data indicating that infections which occurred as late as July 15 did not result in great reductions in yield.

DISCUSSION

Many studies have been made of the active virus content of tomato and tobacco plants, but we have not found any references to studies pertaining to virus content throughout the entire productive life of tomato plants. Following the findings of Alexander (1949, 1950, 1951) that inoculated tomato plants made a recovery and set as many or more fruits per cluster as healthy plants on later clusters, it seemed desirable to follow the active virus content of plants over a period of months. As shown, it could not be demonstrated by the local lesion assay method on *Nicotiana glutinosa* that there was any marked change in active virus content at the time of recovery. It is recognized that this assay method will not detect

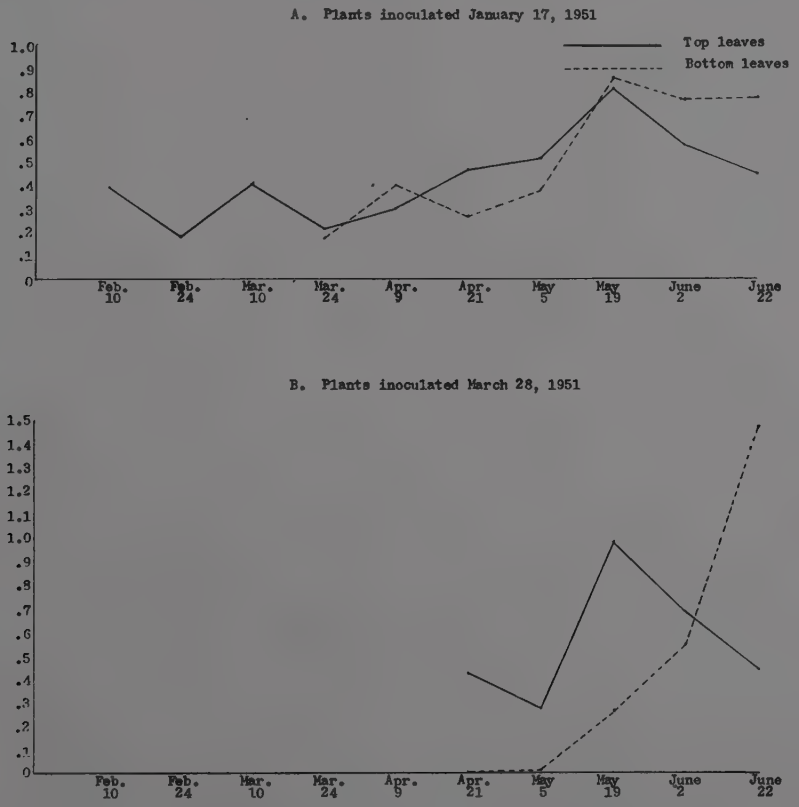

FIGURE 3. Tobacco mosaic virus concentration in leaves of the variety, Ohio W-R Globe, expressed as average number of lesions per square centimeter of half leaves. Sam Dean's Glasshouse. Spring 1951.

small differences (Beale, 1934; Spencer and Price, 1943). However, it was thought that a definite change would be reflected in a curve which was based on biweekly assays.

The curves for virus concentration were erratic and while they tended to show changes, they did not reflect changes at the time of recovery. Following inoculation at early stages, the concentration of active virus was generally uniform throughout the plants. In later inoculated plants the virus concentration tended to remain at a low level. These findings are in accord with those of Samuel (1934) who found that the mature leaves of large plants remain free from virus for as long as three months after the initial inoculation.

In our experiments the inoculations were made on leaves near the growing

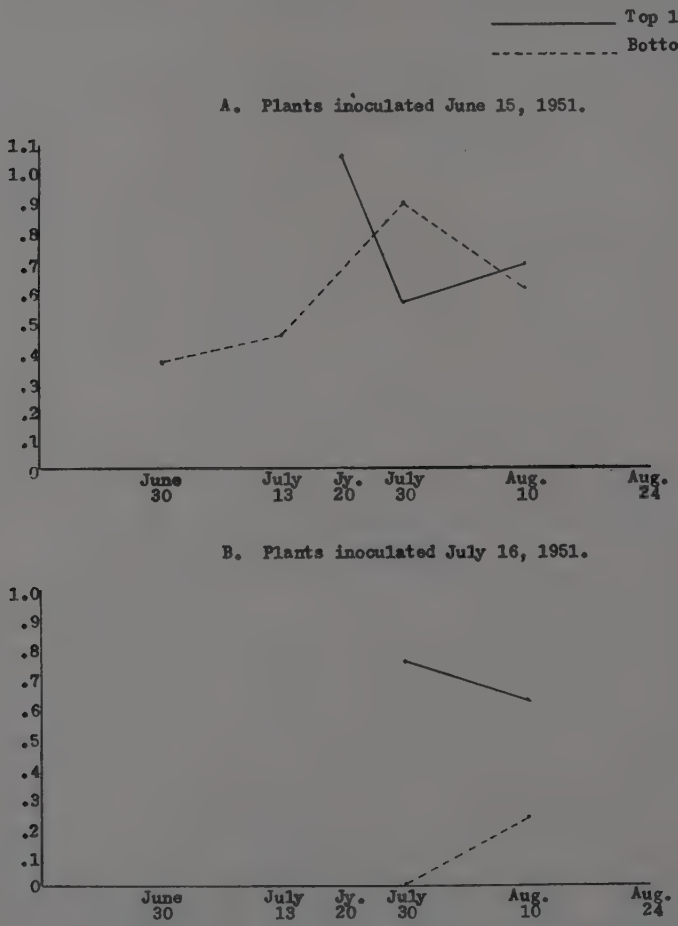

FIGURE 4. Effect of the age of field grown Stokesdale plants inoculated at two different dates on their tobacco mosaic virus content expressed as number of lesions per square centimeter of half leaves. Summer 1951.

point. Kunkel (1939) reported that tobacco mosaic virus moved after at least a 44-hr inoculation period. His findings and those of Capoor (1949) indicated that the virus moves in all directions at about the same time. Thus, other parts of the plants used in these experiments should have received the virus within a few days after inoculation. However, it should be expected that the greatest concentration of virus would be found in the rapidly growing tissue at or near the apex of the plants.

In many instances the active virus content of the plants fluctuated from one sampling period to the next. An unsuccessful attempt was made to correlate these fluctuations with changes in temperature. The effect of nutrition has been shown to have some effect on the virus concentration of plants. Spencer (1935a) reported that virus concentration in leaves could be increased by increased nitrogen supply, and Spencer (1935b) also reported that increased supplies of phosphorous affected the susceptibility of tobacco as the increase benefited plant growth. Bawden (1950) reported that both nitrogen and phosphorous affected susceptibility. The findings of these workers may explain, in part, the fluctuation in active virus concentration of leaves observed in this work because supplementary sidedressings of fertilizer were made at irregular intervals during the growth of these tomato plants.

SUMMARY

The number of tomato fruits set per cluster decreased following infection with the tobacco mosaic virus. Usually the decreased fruit set does not persist beyond the second or third cluster after infection.

There was no apparent relationship between the virus concentration within the plants and recovery of the plants, either from the standpoint of growth or fruit set.

The varieties, Ohio W–R Globe and Strain A Globe, reacted similarly to the virus from the standpoints of virus concentration, a lack of correlation between virus content and return to normal growth, and fruit setting.

The virus concentration of plants inoculated ten weeks after they were set in permanent beds in a glasshouse tended to remain lower than the virus content of plants inoculated at the time of transplanting. This tendency was greatest in the lower parts of the plants.

There was no correlation between the virus content of field-grown, unstaked Stokesdale plants and the recovery of their ability to set fruits.

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TWO NEW SPECIES OF *MELANOTUS*
(COLEOPTERA: ELATERIDAE)

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The two following species of *Melanotus* appear to differ from our described forms.

Melanotus hamatus n. sp.

Male.—Narrow, elongate; dark reddish brown throughout; clothed on both surfaces with pubescence.

Head convex; clypeus obtusely rounded, margined, margin reflexed near eyes, slightly overhanging nasus; surface with coarse umbilicate punctures; antenna densely pubescent, when laid along side extending part of one segment beyond hind angles of pronotum, second segment about as long as wide, third a trifle longer, second and third together shorter than fourth, segments 4 to 10 inclusive strongly serrate; parantennal fovea deep; mandible with a deep elongate fovea near base.

Pronotum slightly longer than wide, widest across hind angles, anterior margin broadly sinuate, median lobe not well indicated; basal margin with deep indentation toward side margin; side margin broadly rounded in front, subparallel in middle, slightly sinuate near base, hind angles acute; disk convex, a slight median depression near base in front of scutellum, a well-developed oblique carina on each hind angle; surface with punctures in middle separated by more than their own diameters, becoming larger and umbilicate toward sides. Scutellum longer than wide, minutely punctate.

Elytra at base narrower than pronotum, widest back of base; sides converging to apices which are rounded to suture; disk convex; surface striately punctate, punctures much coarser toward base, becoming much smaller toward apex, interspaces flat, very finely punctate.

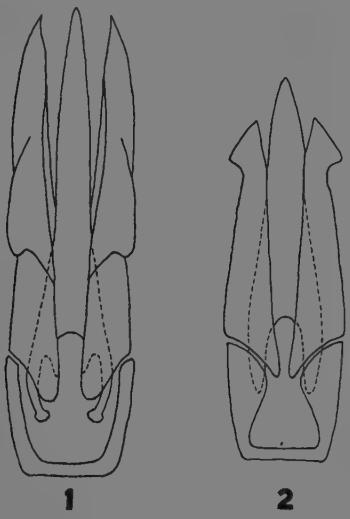
Abdomen beneath coarsely densely punctured toward sides, punctures much smaller in middle, last sternite convex. Prosternum very convex, prosternal lobe declivous, acutely rounded. Posterior tarsus nearly as long as tibia.

Length 10.2 mm; width 2.9 mm.

Female.—Differs from ♂ by antennae not extending to hind angles of pronotum, segments 4 to 10 inclusive less serrate and not as pubescent.

Holotype ♂, allotype and paratype labeled Chisos Mountains, Texas, July 8, 1936, J. N. Knull collector. Paratypes labeled Chisos Basin, Big Bend National Park, Texas, July 16 and 17, 1956, H. and A. Howden and same locality July 4 and 5, 1942, H. A. Scullen. Holotype, allotype and paratypes in collection of author, paratypes in collections of H. A. Howden and M. C. Lane, to whom I am indebted for loan of material.

It can be separated from any of our other species by form of ♂ genitalia (fig. 1).



Ventral surface of male genitalia of:

FIGURE 1. *Melanotus hamatus* n. sp.

FIGURE 2. *Melanotus concisus* n. sp.

Melanotus concisus n. sp.

Male.—Short, narrow, dark brown throughout, legs lighter; clothed on both surfaces with short pubescence.

Head convex, flattened on front, clypeal margin broadly rounded, overhanging nasus; surface with coarse umbilicate punctures; antenna when laid along side, extending part of one segment beyond hind angle of pronotum, second segment about as long as broad, third of about equal size, together subequal to fourth, densely pubescent, segments 4 to 10 longer than wide, serrate; parantennal fovea deep; mandible with deep fovea at base.

Pronotum slightly longer than wide, widest across hind angles; anterior margin broadly sinuate, median lobe broad; side margin broadly rounded posteriorly, subparallel near base, hind angles acute; disk convex a faint prehumeral carina; surface with dense coarse umbilicate punctures on anterior two-thirds and toward sides, basal third finely sparsely punctate. Scutellum elongate, finely punctate.

Elytra back of base slightly wider than base of pronotum; side margin subparallel back of base, then broadly rounded to suture; disk convex; surface coarsely striately punctate, punctures not separated by their own diameters, intervals slightly convex, minutely punctate.

Abdomen beneath finely, sparsely punctate, last sternite densely, coarsely punctured. Prosternum very convex, densely, coarsely punctured; prosternal lobe declivous, margin broadly rounded. Posterior tarsus not as long as tibia.

Length 6.7 mm; width 2 mm.

Female.—Unknown.

Described from a small series taken at light at Artesia, N.M., July 29, 1937 by D. J. and J. N. Knull. Holotype and paratypes in collection of writer. Paratype in collection of M. C. Lane. It can be separated from the other members of the genus by its small size and shape of ♂ genitalia (fig. 2)

THE PRODUCTION OF PENTOSE CONTAINING POLY SACCHARIDES BY *TORULOPSIS FLAVESCENS*

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Although the dextrans have become established as satisfactory "plasma extenders," they do have certain objectionable properties for this particular role. A group of polysaccharides which has not been explored as possible plasma extenders is that of the pentosans, especially those produced by microorganisms. It is conceivable that a pentosan could maintain the blood volume for longer periods of time than a dextran since pentosans should not be so readily hydrolyzed and metabolized by the body. It is also possible that pentosans may lack the antigenic properties of dextrans with certain structures (Kabat and Berg, 1953). In addition, a sugar polymer such as pentosan would represent a more physiological type of substance than compounds such as polyvinylpyrrolidone and presumedly would present less danger of potential toxicity than the latter polymer.

While the above presumptions would require rather extensive investigation, the present work was nevertheless initiated with the object of examining, in a preliminary fashion, the pentose-containing polysaccharides produced by a number of yeasts. Xylose polymers, especially plant xylan, is well-known and has been carefully characterized (Whistler, 1951). In addition, the polysaccharides of green algae (Lewin, 1956), fungi (Martin and Adams, 1956), and pathogenic yeasts (Evans and Theriault, 1953) have been shown to contain pentoses. Since Mager (1947) and Mager and Aschner (1947) reported that a number of non-pathogenic yeasts, especially of the species of *Torulopsis*, produce pentose-containing polysaccharides, various species of this genus were chosen for investigation.

METHODS AND MATERIALS

Strain of organism used.—A number of cultures (kindly provided by Dr. L. J. Wickerham of the Northern Utilization Research Branch, U.S.D.A., Peoria, Illinois) of various species of *Torulopsis* were surveyed for the ability to produce polysaccharides. Four of the cultures investigated produced a polysaccharide material which yielded, upon acid hydrolysis, a component moving similarly to xylose on paper chromatograms. Of these *T. flavescens* NRRL Y 1401 produced the highest yield of polysaccharide and was, therefore, selected for further study.

Culture media. The original screening work was done using a complex medium with the following composition (Juni, 1951); glucose, 45 gm; Difco Proteose-peptone, 3 gm; Difco yeast extract, 3 gm; dibasic potassium phosphate, 4 gm; monobasic potassium phosphate, 4 gm; and distilled water, 1 lit. The pH after autoclaving was 7. The cultures were continuously aerated by incubating on a rotary shaker for 4 days at 30°C. In the later work, it was desired to use media which were synthetic or nearly so and for this purpose, the following were used: *a*) *Modified Wickerham's synthetic yeast medium (1946, 1949).* This medium consisted of 20 ml of Difco Carbon Base (11.7 gm per 100 ml; autoclaved for 15 min); 15 ml of 46 percent glucose solution (sterilized by autoclaving); and 165 ml of a solution consisting of dibasic potassium phosphate, 0.3 gm; Bacto Casamino acids, 0.6 gm; potassium nitrate, 0.16 gm. This solution was also separately sterilized by autoclaving. *b*) *Glucose-Casamino acids-salts medium.* This medium represented a simplification of the above medium and consisted of

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Casamino acids, 3 gm; momobasic potassium phosphate, 4 gm; dibasic potassium phosphate, 3 gm; magnesium sulfate heptahydrate 0.5 gm; trace element solution, 5 ml; and distilled water, 1 lit. The trace element solution consisted of $ZnSO_4 \cdot 7H_2O$, 2.0 gm; $CuSO_4 \cdot 5H_2O$, 0.1 gm; $MnSO_4 \cdot H_2O$, 1.5 gm; $FeSO_4 \cdot 7H_2O$, 2.0 gm and distilled water, 1 lit. After autoclaving of the above medium, 100 ml of 50 percent glucose, separately sterilized, were added. The pH of the medium (minus glucose) was adjusted to 7.0 before autoclaving.

Isolation of polysaccharides.—The method (after Mager, 1947) employed for recovering the polysaccharides from the culture media consisted of the following steps. a) The cultures were centrifuged in order to remove the yeast cells. b) The medium was concentrated on a steam bath and to this concentrate were added 3 volumes of 95 percent ethanol $\left(\frac{v}{v}\right)$ and 10 gm of anhydrous sodium acetate per 100 ml of concentrate. The mixture was then placed in the cold over night at 5-10°C. c) The polysaccharide precipitate was recovered by centrifugation and triturated with 70% ethyl alcohol $\left(\frac{v}{v}\right)$ containing 5 ml of glacial acetic acid per 100 ml. This mixture was centrifuged and the precipitate washed with absolute alcohol, then ether, and allowed to air dry. The product was an amorphous white to slightly tannish powder. This procedure was slightly modified for *T. flavescens* cultures since the latter were highly viscous, making concentration of the medium difficult. The concentration step was omitted, resulting only in the need for using more alcohol and sodium acetate because of the larger volume of culture supernatant. The remainder of the procedure was unmodified.

In order to ascertain the identities of the constituent monosaccharides of the materials isolated, paper chromatographic analyses were applied to the acid hydrolysates of the polysaccharides. Usually, about 50 mg of the polysaccharide were treated with 1 ml of 1.0N sulfuric acid and heated in a boiling water bath for 1 to 2 hr. The mixture was neutralized with barium carbonate and the supernatant obtained by centrifugation was applied to paper chromatograms. Descending development was employed using, as the mobile phase, the organic phase of a mixture of ethyl acetate, acetic acid and water in a ratio of 3:1:3, respectively. The monosaccharides were located on the paper by aid of the spray described by Horrocks and Manning (1949).

Preliminary attempts at fractionation.—As a preliminary attempt to estimate the homogeneity of the polysaccharide materials obtained, the following fractionation procedure was employed. About 250 mg of crude polysaccharide were suspended in 10 ml of 2N HCl, and heated in a boiling water bath until a stable, homogeneous suspension occurred (approximately 5 min). The suspension was cooled and centrifuged, resulting in a small amount of tan precipitate. The latter was designated Fraction A. The supernatant was neutralized to pH 6.5 with 50 percent KOH and a 0.67 volume of ethanol was added. A white precipitate resulted and was recovered. This precipitate was designated Fraction B. To the supernatant, two more volumes of alcohol were added and the precipitate, Fraction C was recovered. All three precipitates were washed respectively in 70 percent alcohol (containing 5 ml of glacial acetic acid per 100 ml), absolute alcohol, ether, and were then air dried.

RESULTS

Conditions for growth and polysaccharide production.—Although the highly complex glucose-proteose-peptone-yeast extract medium was very satisfactory for both growth and polysaccharide production, I felt that a synthetic medium should be devised so that separation of the polysaccharide from other organic materials would be facilitated. In addition, the possibility had to be eliminated that polysaccharides from medium components were contaminating the final isolated product. The modified Wickerham's medium was chosen for this purpose and was found to support excellent growth of the yeast.

In the case of the latter medium, as well as the simplified medium used in later work, the yield of crude polysaccharide was usually about one-fifth of the weight of the glucose in the medium. The polysaccharides were isolated from the supernatant of the culture in the usual manner (without concentration of the medium) and the yield was approximately 1.1 gm from 200 ml of medium. Upon acid hydrolysis followed by chromatography with the ethyl acetate-acetic acid-water solvent, three monosaccharide components could be recognized. One of these gave the R_F of xylose as well as the typical reddish-violet color that the Horrocks-Manning spray gives with a pentose. The other two spots were hexoses, one of which had the apparent R_F of mannose.

Effect of pH on polysaccharide production.—The modified Wickerham's medium was used and the pH was adjusted by the addition of K_2HPO_4 or NaOH to obtain the following: 6.4, 7.0, 7.8, and 9.0. The medium was prepared in 200 ml lots in 500 ml flasks and incubated on the rotary shaker at 30°C for 5 days. At the end of the growth period, the pH's were respectively: 5.0, 6.0, 6.3, and 8.5. Growth was poor at the acid side and excellent at pH 7 as shown in table 1. Polysaccharide yield paralleled the amount of growth. Chromatographic analysis of the hydrolyzates of the polysaccharides showed that at pH 7 and above, the typical picture of two hexose spots and the xylose spot was obtained. At the acid pH however, the slowest moving hexose spot was completely absent (i.e., the one whose R_F corresponds to glucose).

TABLE 1

The effect of pH on growth and polysaccharide production by Torulopsis flavescens*

pH		Relative growth	Total amount of polysaccharide produced (gm)
Before inoculation	After incubation		
6.4	5.0	++	0.23
7.0	6.0	++++	2.26
7.8	6.3	+++	0.96
9.0	8.5	0	0

*Medium: Modified Wickerham's medium, 200 ml per 500 ml flask. Incubated 5 days, 30°C on rotary shaker.

Effect of carbon source on polysaccharide production.—While the modified Wickerham's medium permitted the demonstration that a pentose-containing polysaccharide was produced from glucose by the yeast, this medium was too inconvenient for further study. It was thus necessary to evolve a simpler medium of essentially synthetic composition. Through trial elimination of various components, the glucose-Casamino acids-salts medium was devised and proved to be highly satisfactory for both growth and polysaccharide production.

It next seemed interesting to determine whether polysaccharide production was dependent on the presence of glucose in the medium or whether the yeast was capable of utilizing other sugars for this purpose. The Casamino acids-salts medium was prepared in 45 ml quantities in such concentration that all the ingredients would be in their proper amounts upon the addition of 5 ml of substrate, making a total volume of 50 ml. This was contained in 125 ml Erlenmeyer flasks and incubated on the rotary shaker at 30°C for four days. The substrates used and polysaccharide yields are indicated in table 2.

Effect of glucose concentration on yield.—To study the relationship between polysaccharide yield and glucose concentration, the Casamino acids-glucose-salts medium was used and the glucose concentration was varied between 0.5 and 10 percent, but the pH was kept at 7 in all cases. The results are shown in table 3.

Properties of the polysaccharide(s).—The polysaccharide material isolated from the cultures was a white, amorphous powder which was insoluble in water but did give a rather stable, viscous suspension. It gave a reddish-brown color with iodine and a negative ninhydrin reaction, both before and after acid hydrolysis. The orcinol reaction was strongly positive while the reducing value was very low before hydrolysis and increased after heating with acid (to a degree proportional to the length of heating). The material appeared to be entirely organic (essentially no ash remaining upon ignition). In the growing culture, the polysaccharide material appeared to be a highly fibrous material. When the yield of polysaccharide in the growing culture approached the maximum, the medium became so highly viscous that it poured with difficulty.

The monosaccharides found on chromatograms of acid hydrolyzates of the polysaccharide were found to have R_F values corresponding to glucose, mannose and xylose. The identity of the latter was confirmed by elution of the spot (the edges of which were located with the detection spray) from a chromatogram developed with the ethyl acetate-acetic acid-water solvent and running the eluate on a chromatogram with water-saturated-phenol as the solvent. The unknown had the R_F of xylose with this solvent also.

TABLE 2

The effect of varying the carbon sources on yield of polysaccharide by T. flavescens*

Carbon source	Yield of polysaccharide (gm)**
Glucose	1.96
Xylose	1.48
Mannose	2.18
Mannitol	1.16
Calcium gluconate	0.38
Galactose	1.24

*Medium: Casamino acids-salts mixture plus 0.1% KNO_3 and 1 mg/lit of thiamine HCL; pH was 7. Carbon source were autoclaved as 50% solutions and added to the basal to give a final concentration of 5%. The calcium gluconate was partly in suspension. The volume of medium was 200 ml in a 500 ml flask. Incubated 4 days at 30° C on rotary shaker.

**Chromatographic analysis of the hydrolysates of these polysaccharides showed the same three monosaccharide constituents with the exception of the polysaccharide produced from calcium gluconate, which lacked the component corresponding to glucose.

Fractionation of the polysaccharide(s).—It seemed unlikely that the polysaccharide material isolated would be homogeneous and a number of fractionation procedures were tried in order to isolate fractions of different composition. In one procedure, the material was dissolved in 4 percent NaOH, heated and centrifuged. The supernatant was then fractionated with acetone to yield two fractions, distinctly different in appearance. However, upon hydrolysis, each of the fractions yielded the same three monosaccharide components as did the unfractionated material.

The fractionation method described under Methods and Materials did, however, yield three fractions with different composition, as can be seen in table 4.

Effect of inhibitors on polysaccharide production.—1. Fluoride. Chung and Nickerson (1954) postulated that in the yeast they studied, polysaccharide synthesis proceeded through polymerization of glucose-1-phosphate which was produced from glucose-6-phosphate. If they added fluoride to the growth medium, phosphoglucomutase would be inhibited and polysaccharide synthesis could not occur. This inhibition could be relieved completely by the addition of a small amount of glucose-1-phosphate. In the case of *T. flavescens*, it was found that fluoride

in the concentration of 10^{-2}M also inhibited growth and polysaccharide production completely and the inhibition could be relieved by adding glucose-1-phosphate (0.0077M) simultaneously with the fluoride. The inhibition occurred only when the pH of the medium was on the acid side, presumably due to greater permeability of the fluoride at the lower pH. *2. Other Inhibitors.* The effects of a number of inhibitors on polysaccharide production were determined. The concentration of inhibitor was adjusted to 0.02M with the exception of dinitrophenol which was added in a concentration of 4×10^{-5}M. The glucose-Casamino acids-salts medium was used and the pH was adjusted to 6.0 before autoclaving. The results are shown in table 5. When the concentration of an inhibitor was increased, the degree of inhibition was increased, with the exception of arsenate which was consistently stimulatory to polysaccharide production. The stimulatory effect also occurred when sucrose or maltose was substituted for glucose in the medium.

TABLE 3

The effect of glucose concentration on polysaccharides yield by T. flavescens*

Percent of glucose in medium	Yield of polysaccharide (mg)
0.5	92.4
1.0	126.0
2.5	159.2
5.0	555.8
10.0	566.1

*Medium: Casamino acids-glucose salts; pH was 7. Incubated 5 days at 30° C on rotary shaker. Volume of medium was 50 ml in 125 ml Erlenmeyer flask.

DISCUSSION

One of the most interesting questions that arises in connection with the *Torulopsis* polysaccharides is in regard to the mechanism of synthesis of the xylose and how it is incorporated into the polysaccharide structure. Mager and Aschner (1947) suggested that hexose units might be incorporated into the polysaccharide followed by oxidation of carbon 5 and subsequent decarboxylation, yielding a pentose. However, it was not possible to detect glucuronic acid on the chromatograms under the conditions described. If the latter were present, its concentration was below that of the sensitivity of the chromatographic procedure. In addition, cell suspensions of *T. flavescens* were not able to decarboxylate glucuronic acid in manometric experiments or produce pentose from glucuronic acid. The latter does not, however, eliminate the possibility that an enzyme exists which acts on glucuronic acid when the latter is already a part of the polysaccharide. An alternative approach to the true mechanism may involve such enzymatic reactions as recently described by Avigad, Feingold, and Hestrin (1956), who were able to produce a disaccharide, xylanopyranoxyl-fructofuranoside, by incubating together xylose, raffinose, and levansucrase, or that of Putnam, Litt, and Hassid (1955), who obtained D-glucosyl-D-xylose by incubating glucose-1-phosphate, xylose, and maltose phosphorylase.

What makes it difficult to hypothesize as to the mechanism for pentose incorporation into the polysaccharides is the fact that the latter have not yet been adequately purified and characterized. If it can be found, for example, that *Torulopsis* produces a mixture of pure pentosans and pure hexosans, then it might be reasonable to suppose that there is an enzyme for polymerizing pentoses analogous to the case of hexoses. On the other hand, if the purified polysaccharide can be shown to contain alternating pentose and hexose molecules, one of the

TABLE 4

Alcohol fractionation of polysaccharides produced by T. flavescens

Fraction	Appearance	Water solubility	Yield per gm of starting material	Monosaccharides in acid hydrolyzate as shown by paper chromatography
A	yellowish-tan	insoluble	0.19 gm	glucose; trace of mannose
B	white	soluble	0.55 gm	xylose; small amount of mannose
C	white	soluble	0.04 gm	glucose; mannose and xylose in approximately equal amounts.

previously cited approaches may be valid. Further data for this point must await more adequate fractionation and purification of the polysaccharides.

The data obtained in the inhibitor experiments were the expected results; i.e., those poisons which inhibit either oxidative assimilation or respiration also inhibited growth and polysaccharide synthesis (the latter being dependent on the former, presumably). The one confusing result was the consistently stimulatory effect of arsenate on the polysaccharide production. It is well-established that arsenate can substitute for phosphate (Crane and Lipmann, 1953) in certain reactions and it would seem that arsenate is acting by inhibiting some side reaction which would otherwise remove the substrate for polysaccharide synthesis. The fact that polysaccharide production could be inhibited by fluoride and this inhibition relieved by glucose-1-phosphate would suggest that glucose-1-phosphate is an immediate precursor of polysaccharide, if the interpretation of Chung and Nickerson (1954) to this phenomenon is correct. They postulated that fluoride inhibits phosphoglucomutase, thus interrupting the conversion of glucose-6- to glucose-1-phosphate. The enzyme is presumably protected by the addition of glucose-1-phosphate.

TABLE 5

The effect of several metabolic inhibitors on polysaccharide production by T. flavescens*

Inhibitor	Yield of polysaccharide (mg)
None	327.7
Potassium fluoride	228.3
Potassium arsenite	210.0
Sodium arsenate	433.6
Dinitrophenol	209.8
Sodium azide	170.5
Potassium cyanide	147.1

*Medium: Casamino acids-glucose-salts; pH was 6.0. Volume of medium was 50 ml in 125 ml Erlenmeyer flasks; incubated 5 days at 30°C on rotary shaker. Concentration of inhibitors was 10^{-2}M except dinitrophenol whose concentration was 4×10^{-5}M.

An important objective of this investigation of the polysaccharides produced by *Torulopsis* spp. was to evaluate the suitability of one or more of these polysaccharides as plasma volume extenders. Fraction B (see table 4) appears to be the most promising lead to such a polysaccharide in that this fraction seems to yield essentially pentose upon acid hydrolysis and represents over one half of the weight of the crude polysaccharide.

SUMMARY

Torulopsis flavescens NRRL Y-1401, when grown aerobically, has been shown to produce high yields of polysaccharide material from sugars. The polysaccharides could be separated into three fractions which, upon acid hydrolysis and paper chromatographic analysis, yielded respectively: A, glucose with a trace of mannose and xylose; B, xylose with a small amount of mannose; C, glucose, mannose and xylose in approximately equal amounts.

Polysaccharide production and growth were optimum in a medium of approximately neutral pH. A number of sugars were satisfactory as carbon sources for polysaccharide production, with mannose and glucose giving the highest yields, respectively. Polysaccharide production could be shown to be proportional to the concentration of carbon source (glucose) in the medium with no further stimulation above 5 percent glucose. Polysaccharide production was inhibited by fluoride, arsenite, dinitrophenol, azide and cyanide but stimulated by arsenate. Fluoride inhibition could be completely reversed by the addition of small amounts of glucose-1-phosphate.

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AN INVESTIGATION OF THE HEREDITARY CHARACTER, WOOLLY, IN THE TOMATO[1]

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INTRODUCTION

A condition of very profuse epidermal hair growth exists in some strains of the cultivated tomato, *Lycopersicon esculentum* Mill. The character originated in a field of tomatoes grown by the Campbell Soup Company of Camden, New Jersey and W. S. Porte (personal communication) obtained a seed sample of a tomato having this character from the New Jersey Agricultural Experiment Station. He called the character "Angora." Later, Young and MacArthur (1947), having obtained the selection from Porte, published a description of the character referring to it as both "woolly leaves" and "woolly." Since that time, the character has generally been referred to as "woolly," not as "woolly leaves." Woolly plants are easily distinguished from nonwoolly plants. Due to the profuse epidermal hair growth, woolly plants appear grayish instead of a normal green color.

Upon selfing woolly plants, a ratio of ⅔ woolly to ⅓ nonwoolly plants results. However, when a plant heterozygous for a single gene pair is selfed, either a ¼:½:¼ or a ¾:¼ phenotypic ratio would be expected. Therefore, this observed ⅔:⅓ ratio indicates that the woolly character is lethal when homozygous, and any plant manifesting the woolly character is heterozygous. The woolly character is one of the very few semidominant lethal factors known in cultivated plants, and the nature of the lethal action has not been investigated in any of them.

Since the heterozygotes survive, the woolly gene is not a gametic lethal. If the woolly gene were lethal only to eggs or only to sperms, a ratio of ½ woolly plants to ½ nonwoolly plants would be expected upon selfing a woolly plant, instead of the observed ⅔:⅓ ratio. Therefore, the woolly gene is not lethal in either type of gamete. For this reason, it must be assumed that the lethal action of the homozygous woolly condition takes place sometime after fertilization. If this lethal action occurs during embryonic development, then upon examination of zygotes or embryos in fruits from self-pollinated woolly plants one might be able to observe disintegration of approximately 25 percent more zygotes or embryos than in fruits from nonwoolly plants. This would be 25 percent of all zygotes and embryos, which includes those which are normal and those which are disintegrating.

If the lethal action of the homozygous woolly condition affected embryo or endosperm development and, as a consequence also affected seed coat development, then in fruits from self-pollinated woolly plants, one would expect approximately 25 percent fewer normal sized seeds per fruit than in similar fruits from nonwoolly plants; but nearly the same percent germination should be found in the normal sized seeds from both types of fruits. Conversely, if the lethal action did not appreciably affect seed coat development as a consequence of its effect on embryo or endosperm development, then one would expect nearly the same number of normal sized seeds per fruit in both woolly and nonwoolly fruits but approximately 25 percent less germination of the normal sized seeds from fruits of woolly plants.

According to Soost (personal communication), in seeds germinated on moist filter paper the number not germinating corresponded to the number of expected

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homozygous woolly plants. Dissection of the ungerminated seeds showed that all contained well-developed endosperm while embryos were at various stages of development, but all poorly developed. Soost also found some segregating seed lots which produced a few plants which he suspected were homozygous woolly. Generally these plants did not develop much beyond the cotyledonous stage. If these plants were homozygous woolly, then the lethal action of this genotype is not always fully manifest. The seed used by Soost came from W. S. Porte. Soost, therefore, believed that the lethal action of the homozygous woolly character affected the embryo and not the endosperm and that the exact point of lethal action depended on the genetic constitution of the individual embryo.

Young and MacArthur (1947) suggested that this lethal action could be due to a chromosomal deletion. Although this is a possible explanation, there has been no cytological evidence presented to support such an hypothesis. The chromosome to which the woolly locus is assigned has been variously designated: Young and MacArthur (1947) designated it as chromosome L.; Lesley (1937) designated it as chromosome A.; Barton (1950) designated it as chromosome 2. The primary basis for Barton's classification is chromosome length at pachytene, chromosome 2 being the second longest chromosome. Barton (1951) also stated that the nucleolus is attached to the short arm of chromosome 2. According to evidence presented by Lesley (1937), those genes so far assigned to chromosome 2 are all in the long arm. According to a recent chromosome map (Rick and Butler, 1956), the woolly locus is at 48 crossover units, placing it near the midpoint of the long arm of chromosome 2, there having been 77 crossover units determined. Thus search for a deletion would be narrowed to a small region of chromosome 2.

The woolly character has been described as affecting all vegetative plant parts (Rick and Butler, 1956). Since epidermis also covers reproductive parts, one might expect to find the woolly character affecting reproductive parts as well.

In all strains of another species of tomato, *Lycopersicon hirsutum* Humb. and Bonpl., there is also a condition of profuse epidermal hair growth, for which condition the specific name *hirsutum* has been applied. Plants of this species have epidermal hairs which are much longer than those on plants of *L. esculentum*, whether woolly or nonwoolly, as can be seen in figures 21 and 22. If epidermal hair growth in these two species is conditioned by different genes, upon crossing a *L. esculentum* plant with a *L. hirsutum* plant, offspring might result which had the combined epidermal hair growth of both parents and would then be "super-woolly." There is no record of lethal action associated with *L. hirsutum* type hair.

Therefore, five questions are considered here: *1.* Do irregularities in development occur which can be attributed to lethal action of the homozygous woolly condition? *2.* Is there a difference between fruits of woolly and nonwoolly plants in number and germination of normal sized seeds? *3.* Do woolly plants possess a chromosomal deletion? *4.* Are parts other than leaves also woolly? *5.* What kind of hair condition results from a combination of the woolly condition with the hirsute condition?

EXPLANATION OF FIGURES IN PLATE I

1. Embryo sac of type not classified because it could not be determined whether fertilization had occurred. 461×.
2. Collapsed embryo sac. The stage of embryonic development prior to collapse could not be determined. 461×.
3. Embryo sac with a normal zygote and endosperm. Zygote upper left. 461×.
4. Embryo sac with a normal embryo and endosperm. Two-celled embryo stage. Embryo upper left. 461×.
5. Embryo sac with a normal embryo and endosperm. Many celled embryo stage. 461×.
6. Embryo sac with a normal embryo and endosperm. Many celled embryo stage. 461×.
7. Embryo sac with a disintegrating zygote and endosperm. 461×.
8. Embryo sac with a disintegrating zygote and endosperm. 461×.

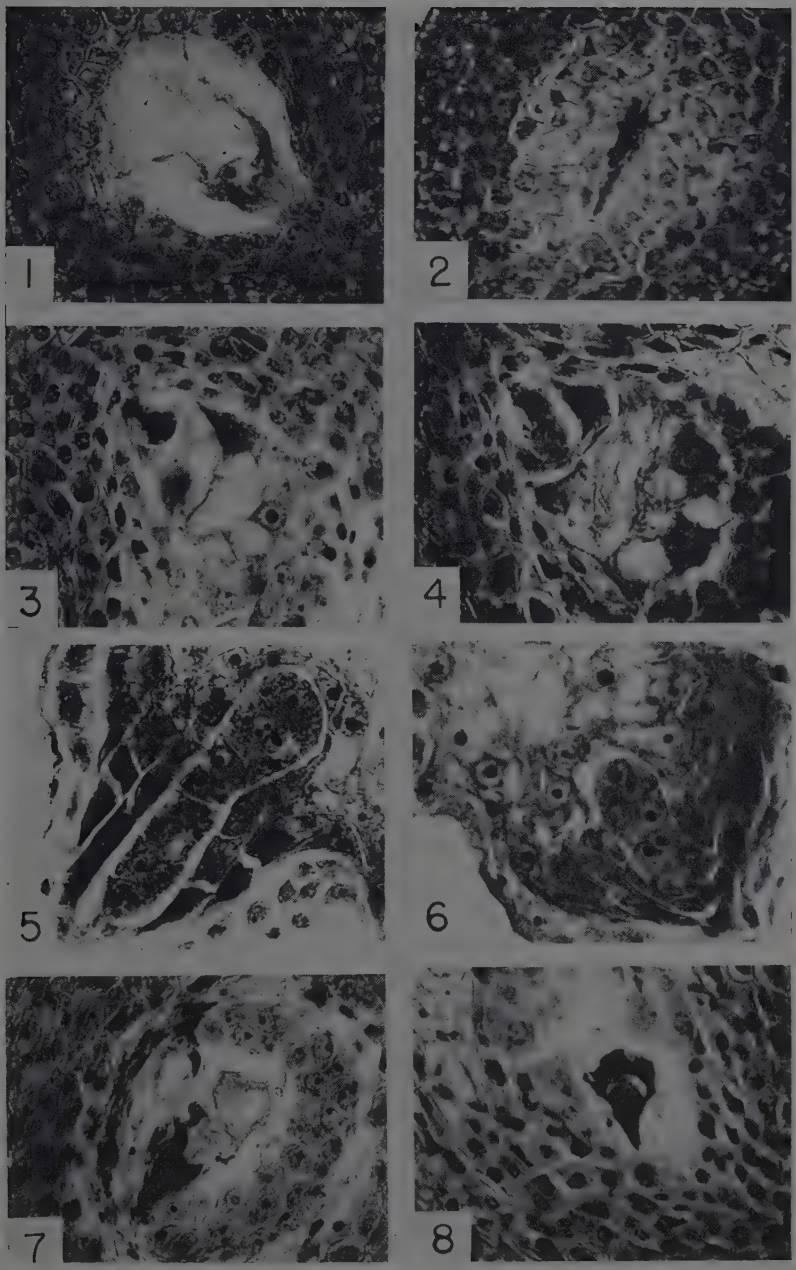
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TABLE 1

Examination of zygotes or embryos in 2347 embryo sacs of 16 fruits of woolly plants

Fruit number	Interval between pollination and removal of fruit, in hours	Stage of embryonic development*	Number of embryo sacs examined	Number of embryo sacs showing disintegration therein	Number of normal embryo sacs	Percent of embryo sacs showing disintegration therein
Class I						
49–3	131	Z.	125	22	103	17.6
16 b	139	Z.	239	35	204	14.6
16 b 4	139	Z.	173	50	123	28.8
56	141	Z.	121	42	79	34.7
20 b 2	143	Z.	186	54	132	29.3
20 b 3	143	Z.	184	28	156	15.2
Total			1028	231	797	
Mean			171±18	39±5	133±18	22.5±3.5
Class II						
49–2	131	2C, Z	100	27	73	27.0
49–4	131	2C, Z	133	28	105	20.9
50–2	139	4C, 2C, Z	142	22	120	15.5
49	131	4C, 2C, Z	120	10	110	8.3
50	139	4C, 2C, Z	80	22	58	27.5
Total			575	109	466	
Mean			115±11	22±3	93±12	19.0±3.7
Class III						
44–3	150	2C	141	33	108	23.4
40	146	2C, 4C	200	34	166	17.0
44	150	emb	95	30	65	31.6
44–2	150	emb	135	65	70	48.1
44–6	150	emb	173	39	134	22.5
Total			744	201	543	
Mean			149±18	40±6	109±19	27.0±5.4

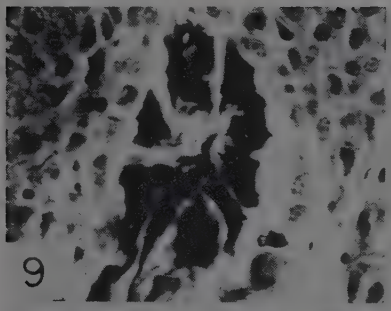
*Z = zygote, 2C = 2-celled embryo, 4C = 4-celled embryo, emb = embryos more than 4 celled.

MATERIAL AND METHODS

1. Embryo sac study.—Woolly and nonwoolly tomato plants for this study were set in the field in the spring of 1951. All were progeny by selfing from one woolly plant. However, the plants were heterozygous for many factors such as fruit color and potato leaf. From August 30 to September 3, 1951, flowers of both woolly and nonwoolly plants were emasculated, self pollinated immediately,

EXPLANATION OF FIGURES IN PLATE II

9. Disintegrating zygote and endosperm. Synergids have also disintegrated. 461×.
10. Embryo sac with a normal zygote and disintegrating endosperm. Synergids have already disintegrated. Zygote upper right. 461×.
11. Embryo sac with beginning zygote disintegration. Normal endosperm. Zygote lower center between and above two darker stained cells. 461×.
12. Embryo sac with beginning zygote and endosperm disintegration. Zygote upper center between two other cells. 461×.
13. Embryo sac with disintegration of a four-celled embryo. These cases were rare. Normal endosperm. 461×.
14. Embryo sac with disintegration of a two-celled or larger embryo. These cases were rare. 461×.
15. P.M.C. showing possible evidence of a heterozygous deletion as indicated by arrow. Note clarity of spindle fiber attachment point in darkly stained portion near nucleolus. 1036×.
16. P.M.C. showing no evidence of a heterozygous deletion. Region where expected is indicated by arrow. This is same cell as figure 15 but at a different focal plane. 1036×.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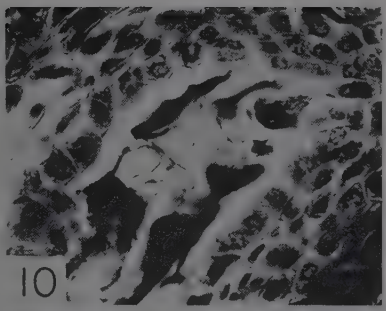
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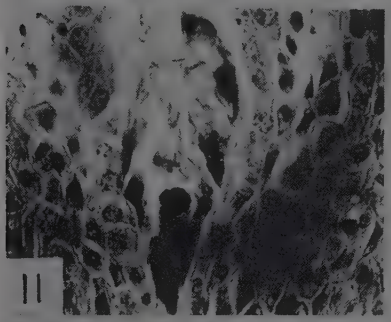
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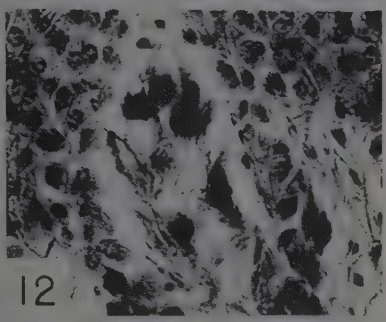
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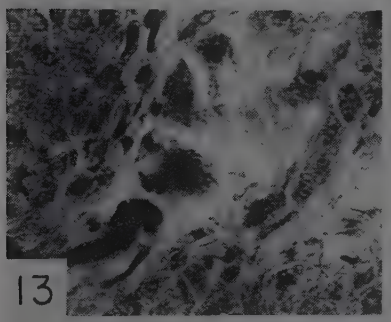
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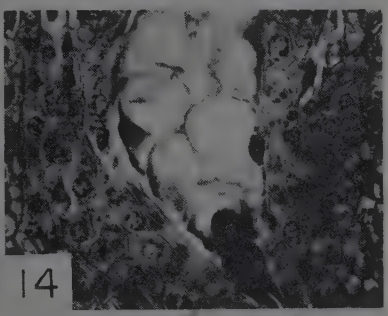
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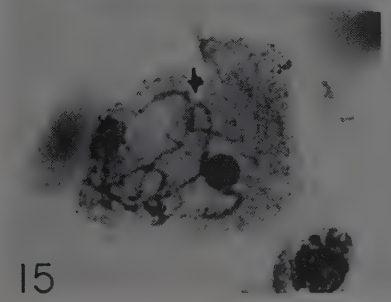
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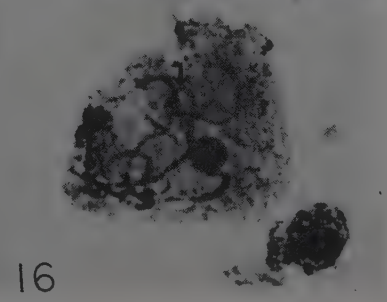
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TABLE 2

Examination of zygotes or embryos in 678 embryo sacs of 6 fruits of nonwoolly plants

Fruit number	Interval between pollination and removal of fruit, in hours	Stage of embryonic development*	Number of embryo sacs examined	Number of embryo sacs showing disintegration therein	Number of normal embryo sacs	Percent of embryo sacs showing disintegration therein
Class I						
19 b	145	Z	82	15	67	18.3
19 b 2	145	Z	90	20	70	22.2
19 b 3	145	Z	131	15	116	11.4
48	142	Z	126	35	91	27.8
22 b	119	Z	134	11	123	8.2
Total			563	96	467	
Mean			113±11	19±4	93±11	17.1±3.5
Class II						
41	150	Z, 2C, 4C	115	43	72	37.3

*Z=zygote, 2C=2-celled embryo, 4C=4-celled embryo.

and bagged with at least a double layer of a fine mesh cheesecloth. All unopened and small flowers were removed from the flower cluster before bagging. From September 3 to September 7, 1951, fruits of both woolly and nonwoolly plants were removed for sectioning at intervals from 80 to 150 hours after pollination.

Serial sections of the fruits were examined for zygotic and embryonic development. Embryo sacs which contained normal zygotes or embryos and endosperm (fig. 3 to 6) and those which showed disintegration of the zygote, embryo, or endosperm (fig. 2 and 7 to 14) were counted.

2. Seed study. Ten fruits per plant were taken from 18 woolly plants and from ten nonwoolly plants in August, 1951. The fruits were cut open and all normal sized seeds were removed and counted.

Seeds were collected and saved from ten fruits per plant of ten woolly and ten nonwoolly plants in September, 1951. Some but not all of the fruits were from the same plants from which seed counts were taken. Seeds from each plant were composited. In December, 1951, germination tests of 50 seeds from each woolly and each nonwoolly plant were conducted on moist filter paper in Petri dishes.

3. Examination of pollen mother cell smears. Acetocarmine smears of pollen mother cells from anthers of greenhouse grown woolly plants were examined for evidence of a heterozygous deletion in the pachytene stage of meiosis. Since Barton had found that the woolly locus is on the nucleolar chromsome, that chromosome was the only one examined in detail. Some cells were observed in which the nucleolus and most of the nucleolar chromosome were pushed away from the rest of the chromosomes as a result of smearing. Most of the nucleolar chromosome was thereby visible. Since in cells of this type better observations could be obtained, most attention was given to these cells (fig. 15 to 18).

4. Description of the woolly character. The expression of the woolly character was studied in a field planting of tomatoes during the summer of 1951. Similar above ground parts of woolly and nonwoolly plants were compared visually. In addition comparable stems, anthers, and styles were examined microscopically and photographed. Particular attention was paid to the length of epidermal hairs on both woolly and nonwoolly plants and to the parts of woolly plants where profuse epidermal hair growth occurred to observe whether any differences exist between woolly and nonwoolly plants.

5. Hybridization with Lycopersicon hirsutum.—Pollen from a *L. hirsutum* plant was applied to emasculated flowers of woolly and nonwoolly greenhouse grown tomato plants during the early spring of 1951. Seeds from these crosses were planted the same year and the resulting plants along with some *L. hirsutum* plants were transplanted to the field. During the summer of 1951, the plants were visually observed to see whether any had both the longer epidermal hairs of *L. hirsutum* and the shorter hairs of woolly *L. esculentum*. In addition, comparable stem segments of woolly *L. esculentum*, *L. hirsutum*, and the F_1 hybrid were photographed.

RESULTS

1. Embryo sac study.—The data of tables 1 and 2 have been arranged into three classes:

Class I—those fruits with all embryo sacs containing zygotes;

Class II—those fruits with embryo sacs variously containing zygotes, two-celled, and four-celled embryos;

Class III—those fruits with embryo sacs variously containing two-celled, four-celled, and many celled embryos.

The data in tables 1 and 2 indicate significant variation among classes in the total number of embryo sacs examined per fruit, embryo sacs with disintegration, and normal embryo sacs. To control this variable, the data were converted to percent of embryo sacs having disintegration.

In comparing data from fruits of woolly plants in table 1, Class I with data from fruits of nonwoolly plants in table 2, Class I, the difference of 5.4 percent more embryo sacs with disintegration in woolly plants is statistically nonsignificant. The data from fruits of woolly plants in Class II indicate there is a decrease, although probably nonsignificant, in percent embryo sacs with disintegration compared with data from fruits of woolly plants in Class I. Comparing data from woolly fruits in Class III, there is a 4.5 percent increase in embryo sac disintegration over Class I woolly fruits, or a 10 percent increase over Class I nonwoolly fruits. This 10 percent increase is not significant so far as lethal action of the homozygous woolly character is concerned.

The data in table 1 show that the stage of development within the embryo sac was not necessarily associated with the interval of time between pollination and removal of the fruit; e.g., embryos of fruits 50–2, 49, and 50 were at a more advanced developmental stage than those of fruits 56, 20 b 2, and 20 b 3, although there was a greater time interval between pollination and removal of the fruit in the case of fruits 56, 20 b 2, and 20 b 3 than in the case of fruits 50–2, 49, and 50. However, considering the sample as a whole, there was a tendency for fruits removed 131 to 143 hours after pollination to have embryo sacs containing embryos at the zygotic, two or four celled embryonic stage, while fruits removed 146 to 150 hours after pollination tended to have embryo sacs containing embryos at the many celled embryonic stage. The flowers for this part of the investigation were not necessarily at the same stage of development when pollinated. The flowers may have been emasculated as much as a few hours to one whole day prior to the beginning of anthesis. Even if flowers had been pollinated at the same developmental stage, subsequent development would not necessarily occur at the same rate, especially between flowers of different plants. This could, at least in part, account for the fact that disintegration of the embryo was not associated with a particular lapse of time between pollination and removal of the fruit for sectioning.

The data in tables 1 and 2 do not include fruits from the entire range of sampling. Fruits with a time interval of less than 119 hours between pollination and removal of the fruit for sectioning contained primarily embryo sacs within which it is doubtful that fertilization had occurred. A few fruits with a time interval greater than 119 hours between pollination and removal of the fruit for sectioning

contained primarily embryo sacs of this same type, and hence also were not included. Since it had already been assumed that the lethal action of the homozygous woolly character occurs after fertilization, little information concerning this problem would have been gained by observing fruits with embryo sacs in which fertilization had not occurred.

Some embryo sacs were observed within which it could not be determined whether fertilization had occurred. Figure 1 is a typical embryo sac of this type containing two nuclei, one larger than the other. It could not be concluded whether the larger nucleus was the fusion nucleus or the primary endosperm nucleus. The smaller might be the nucleus of the egg cell or that of the zygote. Since it was thus impossible to determine whether fertilization had occurred, embryo sacs at this stage of development were not included in the data presented in tables 1 and 2. In the fruits, the data from which are included in tables 1 and 2, embryo sacs in which fertilization could not be recognized were found mostly at the basal end. Since fertilizations occur over a period of time, the ovules in this part of the fruit were the last to be fertilized.

The data in tables 1 and 2 include collapsed embryo sacs (fig. 2) in which it could not be ascertained whether the embryo sac collapsed prior to fertilization (and hence should not be included here) or after fertilization. A collapsed embryo sac indicates that distintegration of its components has occurred but it does not indicate at which developmental stage this disintegration occurred. A collapsed embryo sac is considered as an advanced stage of disintegration.

Figures 3 to 6 are typical examples of embryo sacs containing normal embryos. Figure 3 shows one at the zygote stage. The nucellus and possibly a small amount of the integument can be seen surrounding the embryo sac. Figure 4 shows an embryo sac containing a two-celled embryo. Several endosperm cells can be observed. Figures 5 and 6 illustrate embryo sacs containing many celled embryos.

Figures 2 and 7 to 14 are typical illustrations of disintegration within an embryo sac. Figure 2 is a collapsed embryo sac of the type previously discussed. The embryo within the embryo sac shown in figure 7 is at the zygote stage, judging by the comparative size of the embryo sac. The zygote and endosperm cannot be distinguished since both are disintegrating.

Figure 8 is similar to figure 7 although possibly the zygote is a little better defined. Both the zygote and endosperm are disintegrating. Figure 9 illustrates an embryo sac containing a zygote, which along with the endosperm is disintegrating. The zygote is probably the cell at the upper center of the embryo sac.

EXPLANATION OF FIGURES IN PLATE III

17. P.M.C. showing possible evidence of a heterozygous deletion as indicated by arrow. 1036×.
18. P.M.C. showing no evidence of a heterozygous deletion. Approximate region where expected is indicated by arrow. 1036×.
19. Style segment of woolly *L. esculentum* on the left comparible with that of nonwoolly *L. esculentum* on the right. Note that the upper portion of the style has few or no epidermal hairs in either case. Note greater profuseness of epidermal hair growth on woolly style. 15×.
20. Anther segment of woolly *L. esculentum* on the left comparable with that of nonwoolly *L. esculentum* on the right. Note that profuseness of epidermal hair growth is nearly the same in both cases. 15×.
21. Stem segment of woolly *L. esculentum*. Compare length of epidermal hairs with those in Figure 22. 15×.
22. Stem segment of *L. hirsutum*. Note length of epidermal hairs as compared with figure 21. 15×.
23. Stem segment of an F_1 plant of a woolly *L. esculentum* x *L. hirsutum* cross. This condition is here named superwoolly. Note presence of both short and long epidermal hairs. Compare with figures 21 and 22. 15×.

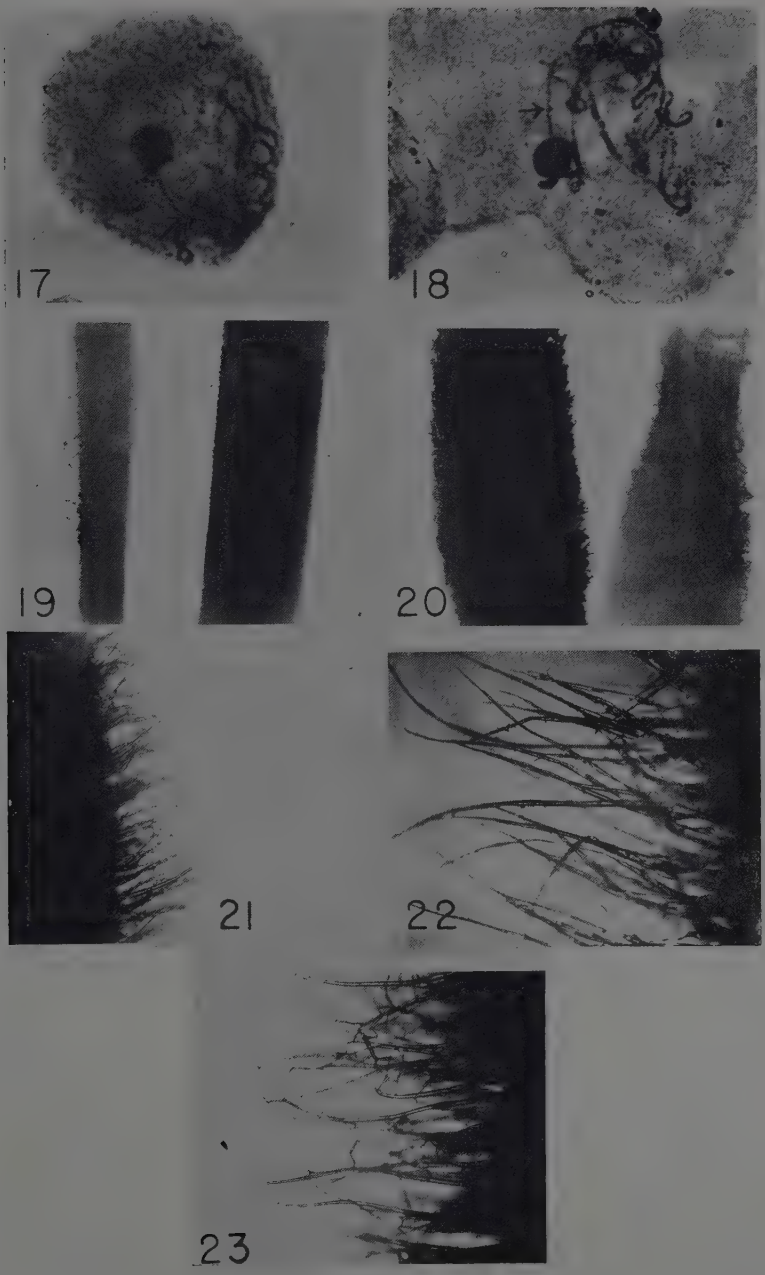
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Figure 10 shows an embryo sac containing a zygote. Several endosperm cells are disintegrating although the zygote, located at the upper right of the embryo sac, still shows a nucleolus but is probably disintegrating. In embryo sacs of nonwoolly plants, 22 such cases were observed while in embryo sacs of woolly plants only three were observed. Compared with the number of embryo sacs observed, these numbers are small. These cases were not considered in computing the amount of disintegration in tables 1 and 2 since it was not certain that the zygote was disintegrating.

Figure 11 illustrates an embryo sac containing a zygote. Endosperm cells are normal. Three other cells are to be noted at the lower center of the embryo sac of which the two darker stained cells might be disintegrating synergids and the third cell, in which the nucleolus is still evident and which is lighter stained, might be the zygote at an early stage of disintegration. Figure 12 shows an embryo sac containing a zygote between two endosperm cells at the upper center of the embryo sac. Two more endosperm cells can also be seen. All cells are at a fairly early stage of disintegration. Figure 13 illustrates an embryo sac probably containing a four-celled embryo which is disintegrating and at least five normal endosperm cells. Figure 14 shows an embryo sac containing a two-celled or larger embryo with at least two normal or nondisintegrating endosperm cells. Disintegration of embryos more advanced than the zygotic stage was rarely seen. In fact, of all the embryos observed to be disintegrating at any stage, only three were composed of two or more cells, two of which were photographed as figures 13 and 14. As is illustrated in figures 11, 13, and 14, in a number of cases in embryo sacs of both woolly and nonwoolly plants, the zygote or embryo disintegrated but the endosperm did not.

TABLE 3 (Summary of Table 6)

Number of normal sized seeds per fruit from 18 woolly and 10 nonwoolly plants, 10 fruits per plant

	Fruits from woolly plants	Fruits from nonwoolly plants
Total seeds counted	17495	9333
Mean number of seeds per fruit	97±4	93±4

2. Seed study.—The number of normal sized seeds per fruit of woolly and nonwoolly plants was nearly the same, as indicated by the data presented in table 3. The mean number of normal sized seeds per woolly fruit was 98±4 and per nonwoolly fruit was 93±4. Since analysis of variance requires the same number of entries per type of sample, data from the first ten woolly plants from which fruits were taken and data from fruits of the ten nonwoolly plants were compared. The results are presented in table 4. The F values obtained are smaller than those required for significance at the 5 percent level. Therefore, it can reasonably be stated that the difference in number of normal sized seeds per fruit from woolly and nonwoolly plants is not statistically significant.

TABLE 4

Analysis of variance of the number of normal sized seeds of 10 fruits of each of 10 woolly and 10 nonwoolly plants

Source of variation	Degrees of freedom		Sum of squares	Mean square	F	$F_{.05}$
Total		199	586655	2948.07		
Between plants	19	19	177083	9320.16	1.010	2.18
Between genotypes	1		11553	11553.00	1.256	247.00
Within genotypes	18		165530	9196.11		
Within plants		180	409582	2275.46	0.247	1.66

TABLE 5

Germination tests of 500 seeds from woolly and nonwoolly plants

Plant number*	Number of seeds germinating per 50
16W	29
17W	31
19W	30
21W	21
23W	39
24W	36
25W	31
26W	26
27W	17
29W	34
Total	294
Mean	29.4±0.68
Mean percent germination	58.8±1.35
13NW	45
15NW	48
18NW	18
19NW	36
20NW	47
22NW	45
23NW	44
25NW	18
26NW	42
32NW	45
Total	388
Mean	38.8±1.15
Mean percent germination	77.6±2.28

Number of woolly seeds expected to germinate = 313

$$\chi^2 = \frac{294-313-.5^2}{313} = 1.215$$

P = .30–.20

*W = woolly plant, NW = nonwoolly plant.

In table 5 data are presented which indicate that 18.8 percent fewer seeds germinated from woolly plants than from nonwoolly plants. Actually, the decrease in germination of seeds from woolly plants is expected to be 19.4 percent rather than 25 percent. A chi-square test of the difference between 18.8 percent and 19.4 percent gave a value of 1.215 with a probability of .30 to .20. Any extrinsic factor which causes some of the seeds from nonwoolly plants to fail to germinate would be expected to cause the same proportion of seeds from woolly plants to do likewise. Any such factor would also be expected to exert its influence independently of the lethal action of the homozygous woolly character. Therefore, some of the decrease in germination in seeds from woolly plants would presumably be due to any factor which causes seeds from nonwoolly plants to fail to germinate plus the lethal action of the homozygous woolly character operating in the same seed. This would make the decrease in germination due to lethal action of the homozygous woolly character appear less than 25 percent by an amount equal to the product of the frequency of factors operating in nonwoolly plants times the frequency of lethal action of the homozygous woolly condition. This has been taken into consideration in arriving at the number of woolly seeds expected to germinate.

3. *Examination of pollen mother cell smears.*—If a deletion were present on one

member of the nucleolar pair of chromosomes, the pachytene configuration would show a hump in one chromosome where it would have no homologous part of the deleted chromosome with which to synapse. The size of this hump would depend on the size of the deletion. If a deletion were not present, the pachytene configuration would be normal, i.e., synapsed throughout. In figure 15 some evidence of a heterozygous deletion might be seen; but in figure 16 which is a photomicrograph of the same cell at a different focal plane, no such evidence is seen. If the deletion hump occurred in a plane perpendicular to the plane being observed, the pachytene configuration would appear normal, excepting that at that point where the deletion occurred, it would appear as though there were a portion of the chromosome which did not stain. Such a configuration is possibly seen in figure 17. Although some cells were observed which show such slight or fair evidence of a heterozygous deletion, many cells were observed (e.g., fig. 18) which showed no evidence of a deletion. If a deletion were actually present, it should be demonstrable in all suitable pachytene preparations from woolly plants.

4. Description of the woolly character.—The woolly character was found to be expressed on most above ground plant parts which included leaves, stems, sepals, petals, parts of styles, and fruits. Below ground plant parts were not investigated. Epidermal hairs are absent from the upper one-third of the styles of both woolly and nonwoolly plants. They are present only on the basal two-thirds of the style in both and are more profuse on styles of woolly plants than on those of nonwoolly plants. Figure 19 illustrates a style segment of a woolly plant on the left and a comparable style segment of a nonwoolly plant on the right. The epidermal hair growth on anthers of woolly plants was not more profuse than that on anthers of nonwoolly plants. This is illustrated in figure 20, which shows an anther segment of a woolly plant on the left and a comparable anther segment of a nonwoolly plant on the right.

5. Hybridization with Lycopersicon hirsutum.—F_1 plants of a species cross involving *L. hirsutum* and woolly *L. esculentum* have both the longer epidermal hairs of *L. hirsutum* and also the shorter epidermal hairs of woolly *L. esculentum*. It is here proposed to designate formally this composite characteristic "superwoolly." Figure 21 illustrates epidermal hairs on a stem of woolly *L. esculentum*. Figure 22 shows epidermal hairs on a *L. hirsutum* stem. From this comparison it may be seen that woolly *L. esculentum* stems had much shorter epidermal hairs than *L. hirsutum* stems. In figure 23, which is from an F_1 plant of a woolly *L. esculentum* x *L. hirsutum* cross, it can be seen that this hybrid had the superwoolly condition. This superwoolly condition occurred on most above ground parts. This indicates that epidermal hair growth of these two species is governed by different genes. Other morphological characters of these F_1 hybrids more nearly resembled *L. hirsutum* than *L. esculentum*.

DISCUSSION

1. Embryo sac study.—No evidence of lethal action of the homozygous woolly character was found within the time range of early embryonic development sampled in this investigation. The only irregularity in development studied here was embryo sac disintegration. Other irregularities may occur at other developmental stages of the embryo, and there may be differential growth rates of woolly and nonwoolly embryos. It is possible that embryos might develop to a certain stage and subsequent development cease as a result of lethal action, which would result in seeds containing poorly developed embryos incapable of germination.

Since, in observing collapsed embryo sacs, it could not be ascertained whether fertilization had occurred, an unavoidable error may have been introduced. However, the number of collapsed embryo sacs which occurred before fertilization would be expected to be the same in both cases. For that reason, the comparison of the amount of disintegration of embryo sacs after fertilization in woolly and non-

TABLE 6

Number of normal sized seeds per fruit from 18 woolly and from 10 nonwoolly plants

Plant number*	Fruit number 1	2	3	4	5	6	7	8	9	10	Total
21W	153	138	128	140	62	14	169	140	69	156	1159
22W	119	129	122	109	38	48	59	30	92	90	836
23W	52	19	100	133	166	106	138	103	143	125	1085
24W	281	17	220	26	170	200	146	147	178	126	1511
25W	88	9	31	34	29	136	127	161	130	185	930
26W	128	135	196	77	157	161	178	142	153	170	1497
27W	134	123	88	112	148	81	136	132	81	119	1154
28W	40	95	92	34	66	100	102	55	37	60	681
32W	115	143	125	18	88	18	134	19	74	97	831
33W	172	74	114	164	142	39	94	165	66	139	1169
34W	54	109	117	28	62	64	27	57	154	130	802
35W	72	39	83	75	113	105	177	43	157	110	974
36W	68	175	43	134	107	87	49	71	70	113	917
38W	66	138	134	56	104	91	107	105	33	145	979
39W	26	164	31	37	60	50	43	77	80	51	649
43W	150	89	107	58	122	107	99	154	115	119	1120
44W	84	60	70	18	53	37	64	81	92	35	594
45W	44	158	76	61	44	37	71	51	57	8	607
Total											17495
Mean											97±4
14NW	37	120	42	37	68	29	54	30	61	45	523
15NW	127	163	37	117	78	60	121	68	100	35	906
16NW	171	194	144	160	111	143	214	127	126	147	1537
17NW	195	125	106	112	129	138	137	98	130	99	1269
18NW	131	80	56	121	53	111	46	126	36	133	893
20NW	103	65	35	76	30	12	35	78	87	85	606
22NW	86	81	20	40	54	92	89	65	110	104	741
23NW	109	12	14	18	124	113	120	102	22	68	702
25NW	20	210	195	21	189	48	136	181	261	24	1285
26NW	47	62	133	81	164	49	85	122	113	15	871
Total											9333
Mean											93±4

*W = woolly plant, NW = nonwoolly plant.

woolly plants would be expected to be a valid approach to the question being considered.

If the cases where endosperm only and neither the zygote nor embryo disintegrated were due to an action of the homozygous woolly gene, one would expect to observe fewer such cases in embryo sacs of nonwoolly plants than in those of woolly plants. The fact is that more were observed and for this reason it was considered permissible to exclude such cases from the data presented.

In a few embryo sacs of both woolly and nonwoolly plants the zygote disintegrated and the endosperm did not. Several factors could account for the zygote disintegration without endosperm doing likewise: *1.* There could be some differential effect of environmental conditions on the embryo and endosperm. *2.* The endosperm might have disintegrated subsequent to the stage observed here. *3.* The triploid endosperm genotype might have been viable even though the diploid zygote genotype was inviable.

2. Seed study.—In view of the significant reduction in germination found in seeds from woolly fruits, lethal action of the homozygous woolly condition must have occurred prior to seed maturity, a mature seed being considered as one capable of germination. Therefore, the lethal action evidently occurred between the many celled embryo stage studied here and seed maturity. It might be pos-

sible that, if lethal action occurred early in the development of the embryo, the endosperm and integument might continue normal development, resulting in normal sized seeds with little or no embryo. However, it seems more plausible that, if lethal action occurred early in embryo development, the endosperm and integument sooner or later might also be affected, thereby resulting in smaller seeds. If this were the case and normal sized seeds of woolly and nonwoolly plants were counted, the number per fruit on an average should be significantly different. This, however, was not observed; and, therefore, it seems reasonable to assume that the lethal action occurred later in embryonic development. If the few plants Soost suspected of being homozygous woolly were actually that, it would indicate that lethal action of this homozygous woolly character occurred late enough in development that the embryos which occasionally escaped this lethal action were then able to germinate. If this is the case, it seems reasonable to say that lethal action of the homozygous woolly character probably occurs shortly before seed maturity.

3. *Examination of pollen mother cell smears.*—On the basis of evidence presented, it is doubtful whether the woolly character is due to a deletion unless the deletion is quite small. However, if the woolly character is due to a deletion, then the expression of woolliness is correlated with the absence of a gene rather than the presence of one.

4. *Description of the woolly character.*—Since epidermal hair growth was found to be more profuse on leaves, stems, sepals, petals, basal two-thirds of the length of styles, and fruits of woolly plants than on those structures of nonwoolly plants, the expression of the woolly character is thus not limited to vegetative parts although it is not always evident on all reproductive plant parts.

5. *Hybridization with* Lycopersicon hirsutum.—The production of superwoolly plants revealed that the genes are different. Whether or not they are alleles is not yet determined. If they are alleles, then neither one is dominant to the other.

SUMMARY

1. No lethal action of the homozygous woolly condition was observed from the zygote stage to the twenty- to thirty-celled embryo stage.

2. The average number of normal sized seeds per mature fruit of woolly plants was very close to that of nonwoolly plants.

3. The average germination of seeds of woolly plants was 25 percent less than that of seeds of nonwoolly plants. This was a significant difference.

4. The lethal action of the homozygous woolly condition probably takes place shortly before seed maturity.

5. It is doubtful that a chromosomal deletion is the cause of the woolly character.

6. The woolly character appears on at least some reproductive parts as well as on vegetative parts.

7. Superwoolly tomato plants were produced by crossing *L. hirsutum* with woolly *L. esculentum*. Hence, epidermal hair growth of these two species must be governed by different genes. If allelic, these genes lack dominance.

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SOME AIR OXIDATION PRODUCTS OF DI-2-ETHYLHEXYL SEBACATE

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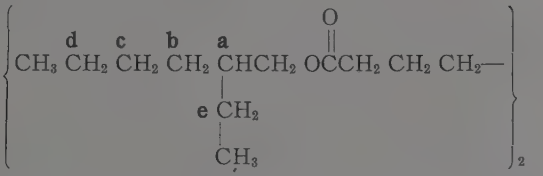
Synthetic lubricants based on diesters are used widely in extreme-temperature military applications (Cohen et al., 1953). The identification of the gaseous oxidation products of such compounds is of interest, in view of a biochemical toxicity attributed to these vapors (Treon et al., 1954). Autocatalytic oxidation (Murphy and Ravner, 1956) and pyrolysis rate (Sommers and Crowell, 1955) studies have been carried out on di-2-ethylhexyl sebacate, a typical such base stock. The former report gave peroxide, acid, carbon dioxide, and water analyses while the latter determined only acid content.

In general, the decomposition of this ester might be expected to follow one of two oxidation paths. In the liquid phase at 50 to 150° C, preferential attack (Walsh, 1946) at the tertiary carbon, **a,** via the usual alkyl hydroperoxide intermediate, should yield mostly ethyl butyl ketone.



In the vapor phase above 200° C, additional attacks (Malmberg et al., 1955) at the various methylene sites will become increasingly important. The anticipated volatile monofunctional carbonyl compounds in the five carbon (C_5) and lower range should be acetaldehyde, **d** and **e**, propionalydehyde, **c**, and butyraldehyde, **b**. A C_2:C_3:C_4 = 2:1:1 degradation proportion would be predicted upon this basis.

EXPERIMENTAL PROCEDURE

A di-2-ethylhexyl sebacate sample (10.000 g) in an all-glass apparatus was heated by means of an electrical controlled salt bath. The contents were continuously air aspirated and the exit vapors were passed into a scrubber containing 2,4-dinitrophenylhydrazone solution. The induction period, defined as the time necessary for cloudiness to appear in the scrubber, and the rate of formation of carbonyl compounds were observed over various temperatures (table 1).

DISCUSSION

The precipitates were collected and were chromatographed (Malmberg, 1954) to determine the nature and relative quantity of the different carbonyl compounds. Each individual 2,4-dinitrophenylhydrazone band assignment was confirmed both by chromatography of known mixtures and by infrared comparison spectra of the eluted derivatives. A zone due to ethyl butyl ketone was present in the two lowest temperature runs, but the quantity produced decreased as the temperature rose. Acetaldehyde, propionaldehyde, and butyraldehyde were found as the predominent products in the last two runs and were present to the proportion of 2:1:1. Acrolein or crotonal derivatives were not observed upon the column.

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These results tend to indicate that the ester decomposes by a predominent liquid-phase oxidation route in the temperature range where it is ordinarily used as a lubricant. However, as the temperature rises, the increasing vapor pressure of the ester leads to larger amounts of small carbon oxidation products.

TABLE 1

Oxidation of di-2-ethylhexyl sebacate as measured by the rate of hydrazone formation

Temperature in °C	Induction period in hr	Total time in hr	Total ppt in mg	Rate in mg/hr
160	2.50	13.0	325	25
190	1.00	6.5	292	45
210	0.42	4.0	266	67
297	0.17	0.9	168	193
333	0.08	0.8	535	640

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LESTES TIKALUS, N. SP. AND OTHER ODONATA FROM GUATEMALA

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Except for general surveys analyzed by Calvert (1901–1908, 1919a, 1919b) there have been no regional studies of Odonata in Guatemala. A small collection which augments knowledge of this area was made between January and May, 1956, as part of an entomological survey in the Department of Petén by Drs. Theodore H. Hubbell, Irving J. Cantrall, and Paul F. Basch, of the University of Michigan Museum of Zoology. A report on Pscoptera (Mockford, 1957) marked the first of a series to appear on the project which was supported by a grant from the Faculty Research Fund of the Horace H. Rackham School of Graduate Studies, University of Michigan.

Most of the specimens were taken in the vicinity of Tikal and Poptún in Petén, and constitute the first survey of Odonata from that area. Williamson's report (1936) on Yucatan dragonflies is the most proximal regional study. The present collection comprises 210 specimens, representing 22 genera and 34 species in six families. Included in the sample are: the plesiallotype female of *Hetaerina pilula;* a holotype male and allotype female of *Lestes tikalus*, new species; a well preserved male of *Gynacantha helenga* which enabled an expanded description of coloration in that species; and two species previously unknown in Guatemala, *Gomphoides sausa pacifica* and *Gynacantha helenga.*

Analysis of the odonate fauna of Petén reveals its tropical American aspects. Of the 34 species recorded here: 19 are widely distributed in Mexico, Central and South America, with 12 of these also occurring in the West Indies; nine are restricted to Mexico and Central America, two to Mexico, Central America and the West Indies, and one to Central and South America; three occur only in Central America. Sixteen of the species have been previously reported and 13 are here recorded, apparently for the first time, from the Yucatan Peninsula.

The vegetation of Petén has been described by Lundell (1937). L. C. Stuart's (MS) description of the Tikal area is the basis for the following notes. Tikal, in the central portion of northeastern Petén, is physiographically situated in the southeastern part of the Yucatan Peninsula on an elevated plateau, the maximum elevation of which is 300 meters. The rolling, poorly drained upland is pitted with depressions; the smaller of these are filled with water during the wet season and are known locally as *aguadas*. The larger depressions, covering many square kilometers, flooded during the wet season and drying out in dry seasons except for occasional aguadas, are termed *akalches* (Mayo) or *bajos* (Spanish). Tikal lies in a luxuriant and deciduous quasi-rainforest in which zapote (*Achras*, chicle tree) is the more important element. In the bajos there is a low forest (canopy 15 to 20 meters high) with considerable light penetrance; the aguadas are typically surrounded by high forest of heavy canopy and contain abundant growths of water plants and tall sedges. Rainfall is estimated to be 160 to 180 cm, with March probably being the driest month. Calvert (1908b) gives the mean annual temperature of Petén as 25° to 30°C.

Aguada Sibal, adjacent to the campsite at Tikal and the source of much of the material reported below, is surrounded by zapotal grading to the east into a bajo. It is about 345 x 255 ft, and estimated to be three ft deep. The aguada is conspicuously well-zoned; on the periphery are several large Bucute trees followed by tall cane (15 ft) and then zones of Mimosa and bullrush, the latter varying 10 to 50 ft in width. Open water is virtually absent due to the abundant growth of floating *Lemna, Spirodela, Wolfia,* and *Pistia.*

Vegetation in the vicinity of Poptún, some 100 kilometers S. S. E. of Tikal in southeastern Petén, is in sharp contrast to the zapotal of the Tikal region, according to field observations of Hubbell and Cantrall. The country is a flat lowland from which arise abruptly many limestone knobs to heights estimated as 400 ft, at most. There is open forest of Caribbean Pine which attains heights of 60 to 70 ft; the meadowlike appearance, with open clumps of low shrubs, gives way to hardwoods to the south. According to the observers, the region, "looks very much like high pine in southwest Georgia." North of Poptún there is a stream bed with steep (2 to 4 ft) banks and some 30 ft wide in which a shallow stream two ft wide trickled in the dry season; southeast there is a drainage way bordered by shrubs and trees.

Minor collections were made in the following localities: Coban, Department Alta Ver Paz; Finca La Paz, Department San Marcos; Panajachel, Department Sololá; Puerto Barrios, Department Izabal; Tiquisate, Department Escuintla. In the list of species, the field collection number appears in parentheses after the abbreviation of the collector (IJC = Irving J. Cantrall; THH = Theodore H. Hubbell; PFB = Paul F. Basch.) Specimens are deposited in the Museum of Zoology of the University of Michigan. I am indebted to Leonora K. Gloyd for determinations of the species of *Argia* and *Argiallagma*, and to Irving J. Cantrall for criticizing the manuscript.

Calopterygidae

Hetaerina cruentata (Rambur). Finca La Paz: in meadowlike cafetal along Buena Vista Creek, 4200 ft, 2 to 2:30 PM, May 3, THH(235), 1♂; along Miraflor Creek, 3600 ft, May 6, THH (254), 2♂. Miraflor Creek is described by the collector as a swift mountain brook, about a foot deep and 3 to 4 ft wide, flowing in a narrow steep valley with heavily vegetated slopes, and with large boulders and pools in the stream.

Hetaerina titia (Drury), "tricolor" wing pattern. Along steep and partly shaded banks of Río San Pedro at gate to military camp on road north of Poptún, April 16, THH+IJC(181), 1♂.

Hetaerina pilula Calvert. Poptún, in small clearing in gallery forest along large pool in Río San Pedro, 8:30 to 10 PM, April 16, THH+IJC(178), 2♂. According to the collectors, the river is a succession of deep (to 10 ft) and clear pools with narrower and swifter reaches, some with rapids of small size over limestone rocks.

This is apparently the first record of this species from the Yucatan Peninsula.

Hetaerina pilula, Plesiallotype Female (fig. 3). A female, collected with the two males of *pilula*, was initially identified as *H. macropus* according to Calvert's (1901) key. It differed from *macropus* in several characters, notably in the presence of two spines on the dorsoapical margin of abdominal segment 10, one on each side. These spines are shorter and more slender than the spine which continues the middorsal carina. Comparison of the female with the description of the male of *Hetaerina pilula* indicated that this was the undescribed female of that species. The specimen was sent to Calvert for an opinion; his reply (August 8, 1957) contains the following: "Your Guatemala female does not differ more . . . from the description of *pilula* male than do females of *macropus* from *macropus* males; bearing this in mind I think it would be safe to call it *pilula*."

The female differs from the description of the male (Calvert, 1901, pp. 33–34) as follows:

Head with nasus with a cupreous metallic reflection, vertex metallic dark green with a coppery-yellow reflection.

Prothorax and thoracic dorsum without coppery-red reflection; a narrow metallic green band along the thoracic carina, widened posteriorly to the humeral suture; a metallic green band on each of the mesepimeron, metepisternum, and metepimeron, that of mesepimeron interrupted posteriorly at three-fourths its length; yellow humeral stripe four to five times wider than the dark brown of the same side of the thoracic dorsum.

Abdomen with a small dorsolateral metallic green spot on segment 1; an elongate dorsolateral green spot extending one-half the length of segment 2; segment 10 with middorsal carina extended and elevated as a slender black spine; two dorsoapical spines on segment 10,

one on either side of the middorsal spine, one-half as long and more slender than middorsal spine; ventrolateral apical spines (2 on left, 3 on right) and ventroapical spines, one on each side, as long as the two dorsoapical spines.

Appendages nearly as long as segment 10, conical, acute; genital valvules extend to ventral apex of tergum of segment 10, apical half of inferior margin denticulated.

Stigma obscure in color, surmounting less than one-half cell on all wings; tip of each wing unicolorous with rest of wing, not brown; coppery-red metallic reflection on longitudinal veins from R_1 to lower sector of arculus and outward to beyond nodus, otherwise no coloring of the wing to represent the coloring of the base of the wing in the male; median cross veins, 6 in forewing, 7 in hindwing (vs. 9 in male); quadrilateral crossveins, 6 in forewing, 5 in hindwing (vs. 7–9 in male); antenodal crossveins, 23 in left forewing, 21 in right forewing, 22 in left hindwing, 23 in right hindwing (vs. 22 in male).

Abdomen, 34 mm; hindwing, 30 mm

The plesiallotype female, labeled, "Guatemala: Department Petén, Poptún, April 16, 1956, I. J. Cantrall and T. H. Hubbell, #178," is deposited in the type collection of the University of Michigan Museum of Zoology.

The following modification in Calvert's key (1901, p. 21) will permit separation of *macropus* and *pilula* females.

f. Pterostigma, if present, obscure in colour.

f_1. A dorsolateral apical spine on each side of spine which continues the middorsal carina.....9. *pilula*

f_2. No dorsolateral apical spine on each side of spine which continues the middorsal carina.....10. *macropus*

Lestidae

Lestes tenuatus Rambur. Tikal: along trail to Great Plaza, April 8, THH+IJC(145), 1♂; Aguada Sibal, April 8, THH+IJC(147), 1♀; around periphery of Aguada Sibal, 3 to 4 PM, April 11, THH+IJC(160), 7♂; along first three-fourths mile of trail to Aguada Naranja in low shrubbery and small trees, May 14, THH(271), 2♂, 1♀; west and south sides of Aguada Sibal in zone to knee-to to thigh-high herbage, 4 to 5:30 PM, May 17, THH(278), 3♂, 1♀. Poptún, in pine savanna at outskirts of city, 2 to 5 PM, April 14, THH+IJC(170), 1♀.

Lestes tikalus, new species.

Figures 1, 2, 4, 5

Holotype male.—Labium brownish yellow, movable spine of palpus black; mandibles yellowish-brown to brown at base, black at tips; labrum pale blue becoming brown; anteclypeus blue becoming black; postclypeus and frons brown to black (a narrow yellow line along dorsoanterior edge of postclypeus in holotype); vertex dark metallic green darkening to black; posterior of epicranium with broad border of dark brown; rear of head becoming pruinose, pale but with considerable black in older individuals, pale yellowish-brown to brown around foramen.

Prothorax becoming wholly pruinose over a yellowish-brown base color. Thorax becoming wholly pruinose, base color brown; middorsal thoracic carina yellow (brown in older individuals and continuous with narrow border of brown); each mesepisternum with a metallic green stripe extending from basal ramus, one-fifth to one-fourth as wide as mesepisternum itself, narrowing posteriorly before abruptly widening ventrally at five-sevenths its length to about one-half the width of the mesepisternum, separated from the middorsal carina and antealar carina by a brown area as wide as the anterior width of the stripe, separated from humeral suture by a pale to dark brown stripe (wider anteriorly) plus pruinose blackish which together are twice as wide as the stripe; mesepimeron pale brown with a centrally located metallic green stripe, roughly triangular in form with the apex posterior, one-half the width of the mesepimeron at base and in older individuals grading imperceptibly into dark brown which also covers the metepisternum posteriorly from the spiracle; metepisternum in younger individuals pale brown, dark brown around the spiracle; an elongate black spot ventrally on both the anterior and posterior end of the metepimeron which is pale brown

anteriorly, dark brown posteriorly. Femora brown, with three black lines which are superior, anterior, and inferior, respectively. Tibiae dark above, pale below; tarsi black.

Wings reaching to middle of abdominal segment 6; membrane hyaline, wing veins and stigma brownish black. Postnodals: forewings, 9–12 on left, 10–11 on right; hindwings, 8–12 on left, 9–11 on right. M stem arises at the lower fourth of the upper limb of the arculus; Rs and M_3 at or slightly distal to level of apex of quadrangle; M_2 at midlevel between third and fourth to midlevel between fourth and fifth postnodal in forewing, at or before third to midlevel between third and fourth in hindwing; anal cross vein at considerably before the midlevel of antenodals in forewing, just or considerably before in hindwing. M_{1a} is zig-zag its full length. M_3 reaches hind end of wing at or beyond level of outer end of stigma in both wings. Stigma surmounts two cells. Cu_2 12–19 cells long in forewing, 10–15 in hindwing.

Abdomen becoming pruinose on segments 1, 2, and 7–10; dark metallic green becoming black dorsally; brown laterally on segments 1–6; segments 7–10 dark brown; segments 1–7 with a basal, dorsally-interrupted transverse pale blue ring, 2–6 with a lateroapical pale blue spot which becomes black. Pattern otherwise obscure.

Superior appendages 1.5 times as long as side of segment 10, two-thirds the length of segment 9; brown at base, black apically. In dorsal view, appendages curved toward each other in their distal halves, distal margin of outer edge denticulate; inner edge of each appendage bears at one-fifth its length an acute tooth directed mesocaudad followed by a lamina with an almost straight nondenticulated margin for about one-fourth the length of the appendage, the lamina terminating in an acute caudomesad-directed spine, which is succeded by a concavity, and this in turn by a small rounded triangular protuberance at two-thirds of the appendage length; distal half of appendage bearing a dorsal groove on the proximal three-fourths. In profile view, the proximal two-thirds almost straight, the distal third curved downward.

Inferior appendages nearly or fully as long as superiors, brownish-black, uniformly curved toward each other. In profile view, the proximal two-fifths wider than distal three-fifths.

Abdomen (including appendages), 31.3 mm (29.5–33.0); hindwing, 19.9 mm (19.0–20.5); stigma, 1.3 mm.

Described from five males from Guatemala as follows: Department Petén, Tikal, March 7, 1956, I. J. Cantrall, No. 101, 1♂ (HOLOTYPE); Department Petén, Uaxactún, May 6, 1931, A. Murie, 2♂; Department Izabal, Los Amates, June 22, 1909, E. B. Williamson, 2♂. The specimens are deposited in the University of Michigan Museum of Zoology except for one paratype male from Uaxactún which is deposited in the Academy of Natural Sciences of Philadelphia. The specimens from Uaxactún and Los Amates were found among a series of undetermined *Lestes* in the UMMZ.

Allotype female. Head as in male except labrum black, clypeus brown and lacks yellow stripe.

Prothorax as in male. Thorax color pattern as in male except as follows: middorsal thoracic carina pale, not bordered by black; mesepisternum lacking pruinose black area; mesepimeron and metepisternum lacking dark brown except around spiracle.

Wings as in male except as follows: postnodals in forewings, 10 on left, 11 on right, and in hindwings, 10 on left, 11 on right; M_2 arises just beyond midlevel of third and fourth postnodal in forewing, just beyond third postnodal in hindwing; M_3 reaches hind end of wing at level of middle of stigma in hindwing; Cu_2 in forewing 16 (left), 17 (right) cells long, in hindwing 14 (left), 13, (right).

Abdomen with slight prunosity on segments 1 and 8; slight metallic green reflection on segments 2–4, otherwise brownish-black; basal rings on segments 3–7 pale (blue?); segments 2–5 with lateral apical pale (blue?) spot. Appendages brownish-black.

Ovipositor black with brown area dorsad on base of outer valves; cerci as long as side of segment 10; valves extend as far as the extreme end of the abdomen; posterolateral margins of basal plate of ovipositor angulate, the angle produced into an acute process almost as long as length of basal plate.

Abdomen (including appendages), 29 mm; hindwing, 20.3 mm; stigma, 1.3 mm.

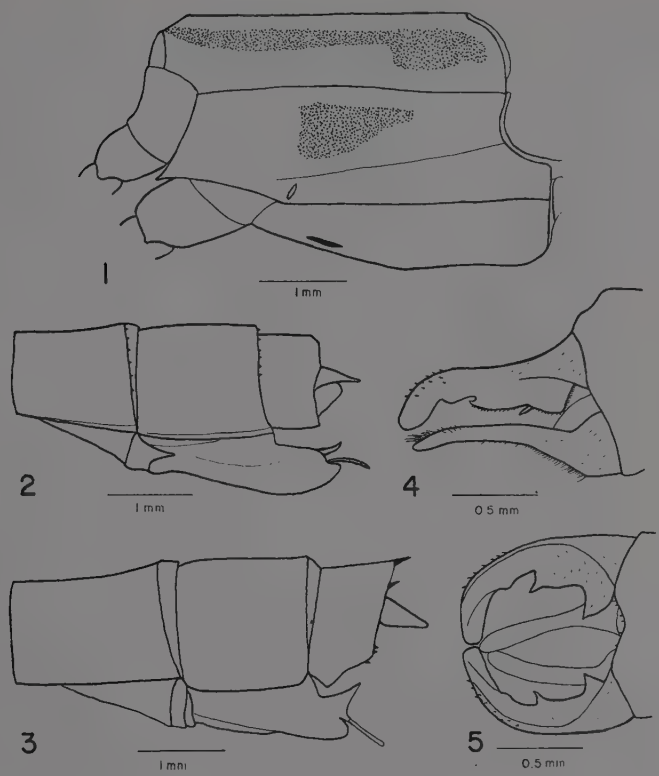

FIGURE 1. Pterothorax color pattern of allotype female of *Lestes tikalus*, n. sp.
FIGURE 2. Abdominal segments 8–10 of allotype female of *Lestes tikalus*, n. sp.
FIGURE 3. Abdominal segments 8–10 of plesiallotype female of *Hetaerina pilula* Calvert.
FIGURE 4. Lateral view, anal appendages of holotype male of *Lestes tikalus*, n. sp.
FIGURE 5. Superior oblique view, anal appendages of holotype male of *Lestes tikalus*, n. sp.

Described from a single female labeled, "Guatemala: Department Petén, Tikal, February 20, 1956, I. J. Cantrall, #59," and deposited in the type collection of the University of Michigan Museum of Zoology.

The female is considered to be correctly associated with the male because of the similar peculiarities of the color pattern of the thorax, venation of the wing, and capture in the same locality (campsite at Tikal).

The relationships of *Lestes tikalus* are those with typical species of the genus. The male superior appendages are nearest *Lestes scalaris* (Calvert, 1909, p. 94 and Pl. I, fig. 18) in regard to the inner edge except that the lamina in *tikalus* terminates in an acute spine much as in *L. mediorufus* (Calvert, 1909, pp. 97–98 and Pl. II, fig. 24). The inferior appendages in *tikalus* are as long as the superiors, whereas in *scalaris* they are noticeably shorter. The thoracic color and color pattern of *tikalus* is different from *scalaris*, but grossly similar to that in *forficula* (Klots, 1932, p. 77; Clavert, 1928, pp. 5–8; and when compared with a series of 13 males in the University of Michigan Museum of Zoology collection including 2 from Cuba, 2 from Mexico, 3 from Jamaica, and 6 from the Dominican Republic). In *tikalus* the mesepisternal stripe is abruptly widened ventrally to twice its width at the upper end whereas in *forficula*, widening, if present, is slight and uniformly gradual; the mesepimeron stripe is

two to three times wider than that in *forficula*: inner edge of appendages of the male *tikalus* bears a second spine as long as the basal one, with the intervening lamina smooth whereas in *forficula* there is no second spine and the lamina is denticulated.

The following modifications in Calvert's key (1901, p. 48) will permit separation of *Lestes tikalus* from *L. forficula*:

aa. Each mesepisternum with a metallic green stripe one-fourth to one-sixth (or less) as wide as mesepisternum itself, upper end usually widened.

aa$_1$. Upper end of mesepisternal green stripe not abruptly widened, if at all; mesepimeron stripe narrow, one-eighth to one-tenth as wide as long; superior appendages of male with an acute basal tooth and a convex median dilatation on inner side, the lamina of which is denticulated; inferiors almost as long as the superiors, reaching beyond the level of the apex of the median dilatation thereof.....4. *forficula*

aa$_2$. Upper end of mesepisternal green stripe abruptly widened to about twice its width; mesepimeron stripe roughly triangular in form, about as wide anteriorly as long; superior appendages of male with an acute basal tooth and a median dilatation on the inner side, the lamina of which is non-denticulated and terminates in an acute tooth as long as the basal one; inferiors as long as the superiors.....18. *tikalus*

The habitat of *Lestes tikalus*, based on the tikal material, appears to be aguadas (see description of Aguada Sibal *antea*) in lowlands and coastal plains (Los Amates).

Coenagrionidae

Argia extranea (Hagen). Finca La Paz, in cafetal near Santa Teresa Creek, 3500 feet, May 3, THH, 1♂. Panajachel: on shore of Lago Atitlan, January 20, IJC(3), 2♂; in cafetal and along small irrigation ditches, 10 to 12 AM, April 29, THH(213), 1♂, 1♀.

Argia fissa Selys. Coban, on grass and low leafy shrubs, May 1, PFB(1), 1♀.

Argia frequentula Calvert. Poptún: April 16, THH+IJC(178), 2♂, 2♀ (see *Hetaerina pilula*); in monte, along road from Poptún to San Luis, April 20, THH+IJC(196), 4♂, 1♀.

This is apparently the first record of this species from the Yucatan Peninsula.

Argia oculata Hagen. Poptún, in gallery forest along Río San Pedro, April 16, THH+IJC(1,7), 1♀.

This is apparently the first record of this species from the Yucatan Peninsula.

Argia ulmeca Calvert. Poptún: April 16, THH+IJC(180), 1♂ (same as 178, see *Hetaerina pilula*); April 16, THH+IJC(181), 1♂ (see *Hetaerina titia*).

Argiallagma minutum (Selys). Tikal: in a zone of *Panicum* on west edge of aguada at camp, February 20, IJC(58), 1♂; adjacent to aguada at camp, February 20, IJC(59), 4♂, 3♀; at Aguada El Naranjo, April 11, THH, 2♂.

This is apparently the first record of this species from the Yucatan Peninsula.

Anisagrion allopterum Selys. Panajachel: January 20, IJC(3), 1♀ (see *Argia extranea*); in sunny open corner of cafetal, April 28, 1560 meters, THH(208), 1♂; April 29, THH(213), 3♂, 3♀ (see *Argia extranea*).

Anisagrion lais (Brauer). Panajachel, April 28, THH(208), 1♂ (see *Anisagrion allopterum*).

Enallagma coecum novae-hispaniae Calvert. Poptún, April 16, THH+IJC(181), 1♂ (see *Hetaerina titia*).

This is apparently the first record of this species from the Yucatan Peninsula.

Telebasis filiola (Perty). Tikal, April 11, THH+IJC(160), 2♂, 1♀ (see *Lestes tenuatus*).

Telebasis salva (Hagen). Tikal, April 11, THH+IJC(160), 1♂ (see *Lestes tenuatus*).

Leptobasis vacillans Selys. Tikal: resting on vegetation along edge of Aguada Sibal, February 15, IJC(49), 2♂; February 20, IJC(58), 1♂, 1♀ (see *Argiallagma minutum*); February 20, IJC(59), 1♂, 1♀ (see *Argiallagma minutum*); Aguada Sibal, April 8, THH+IJC(147), 1♀ (see *Lestes tenuatus*); April 11, THH+IJC(160), 10♂, 8♀ (see *Lestes tenuatus*); May 17, THH(278), 2♀ (see *Lestes tenuatus*).

Ischnura ramburii (Selys). Tikal: at lamp-light at table in camp, April 10, THH+IJC(100), 1♂; in guarumal south of road from camp to airstrip, collecting by headlight, 7 to 10 PM, April 12, THH+IJC(164), 1♀.

Anomalagrion hastatum (Say). Puerto Barrios, in center of town on a low carpet-grass covered fill over a former mangrove swamp, January 30, IJC(20), 1♀. 5.4 miles south of Puerto Barrios, sweeping at night, February 1, IJC(21), 1♂. Coban, May 1, PFB(1), 1♀ (see *Argia fissa*).

Ceratura capreola (Hagen). 5.4 miles south of Puerto Barrios, February 1, IJC(21), 1♂, 1♀ (see *Anomalagrion hastatum*).

Gomphidae

Gomphoides sausa pacifica Selys. Poptún, April 14, THH+IJC(170), 1♂ (see *Lestes tenuatus*).

This is the southernmost record of this subspecies previously unrecorded in Guatemala; Calvert (1919) reported *G. s. sausa* in Guatemala.

Aeshnidae

Aeshna (*Hesperaeschna*) *psilus* Calvert. Finca La Paz, Municipio de la Reforma, in house, May 3, THH, 1♀.

Triacanthagyna septima Selys. Tikal: flying at dusk near aguada, February 13, IJC, 1♀; flying at dusk around camp, April 8, THH+IJC(101), 1♀.

This is apparently the first record of this species from the Yucatan Peninsula.

Triacanthagyna ditzleri Williamson. Tikal, about dusk around camp, May 16, THH(274), 1♀.

Williamson (1923) gives the maximum length of the stigma in the front wing as 3.6 mm; in the female from Tikal that length is 3.9 mm although other mensurable features are as stated in Williamson's description. The Tikal specimen is apparently the northernmost record for this species, and the first record of it from the Yucatan Peninsula.

Gynacantha helenga Williamson and Williamson. Poptún, April 16, THH+IJC(178), 1♂ (see *Hetaerina pilula*).

This species is not previously recorded from Guatemala or the Yucatan Peninsula; it has been known only from the type locality in Jalisco, Mexico. The excellent color preservation on this male, superior to that of the type male with which it has been compared, permits the following additions and corrections to the original description (Williamson and Williamson, 1930). The clypeus and frons are bluish green rather than brownish green; on either side of the T-spot on the dorsum of the frons, the color is pale blue as in *G. tibiata* Karsch, rather than clear green; the dorsoposterior angle of the metepimeron is brownish blue; metacostal process is pale blue; apical half of dorsum of abdominal segment 1 is blue rather than "light brown?"; the anterior third of abdominal segment 2 is brownish green except for a basal blue annulus, and as stated by the Williamsons, the posterior two-thirds is blue with brown stripes. The tubercle in the median line near the apex of the sternum of abdominal segment 1 bears a well-marked depression on the ventral surface, similar to that in *G. tibiata*.

Gynacantha nervosa Rambur. Tikal: at dusk near aguada, February 13, IJC, 1♀; hawking at dusk around eaves of champa, April 7, THH+IJC(101), 1♀; May 16, THH(274), 6♂, 6♀ (see *Triacanthagyna ditzleri*); at camp grounds, May 18, THH(101), 2♂, 2♀; flying at dusk around camp, May 19, THH(101), 5♂, 2♀; at camp grounds, May 20, THH(101), 2♂, 1♀.

This is apparently the first record of this species from the Yucatan Peninsula.

Libellulidae

Uracis imbuta (Burmeister). Tikal, along first 1.5 miles of Uaxactún trail which is bordered by medium tall and low shrubbery and small trees, 1:30 to 5 PM, May 19, THH(282), 1♂.

Micrathyria didyma didyma (Selys). Tikal: at campground, March 26, IJC(101), 1♀ (teneral); at table in champa at camp, April 1, IJC(101), 1♀ (teneral); along trails to Great Plaza, Pyramid 2 and aguada north of Pyramid 2, 2 to 5:30 PM, May 18, THH(281), 1♂; May 19, THH(282), 1♂ (see *Uracis imbuta*).

Anatya normalis Calvert. Tikal: collecting by headlight in brushy area at north temple, 7 to 9 PM, April 7, THH+IJC(141), 1♂; April 11, THH+IJC(160), 1♂ (see *Lestes tenuatus*); May 14, THH(271), 1♀, (see *Lestes tenuatus*); May 17, THH(278), 1♀ (see *Lestes tenuatus*); May 19, THH(282), 1♀ (see *Uracis imbuta*).

This is apparently the first record of this species from the Yucatan Peninsula.

Erythrodiplax umbrata (Linnaeus). Puerto Barrios, at night, February 1, IJC(22), 1♂ (same as 20, see *Anomalagrion hastatum*). Tikal: April 7, THH+IJC(141), 1♀ (see *Anatya normalis*); in bajo in a small sunny opening around young escobas and palm fronds, April 9, THH+IJC (150), 3♂, 2♀; in great plaza of ruins, May 13, THH, 1♂; at east end of airstrip at which taller forest trees were felled in the A.M., collecting by lantern and headlights on foliage of felled trees, 6 to 9:30 PM, May 17, THH(279), 1♂, 3♀; May 19, THH(282), 1♂, 1♀ (see *Uracis imbuta*); around camp, May 20, THH, 1♀. Poptún: April 14, THH+IJC(170), 2♂, 2♀ (see *Lestes tenuatus*); collecting by headlights in pinewoods north of Poptún, 7:30 to 10:30 PM, April 15, THH+IJC(172), 19♂, 14♀ (same as 170, see *Lestes tenuatus*); April 16, THH+IJC(178), 2♂, 2♀ (see *Hetaerina pilula*).

Except for two homochromatic females taken at Poptún, April 15, all of the females are of the heterochromatic form.

Erythrodiplax funerea (Hagen). Tiquisate, in banana plantation, 10 kilometers from coast near bend in Zanjon del Mico, 1 to 4 PM, May 8, THH(257), 1♂.

Dythemis velox Hagen. Poptún: April 14, THH+IJC(170), 1♂ (see *Lestes tenuatus*); along creek north of town, April 15, THH+IJC(175), 2♂; April 16, THH+IJC(178), 1♂ (see *Hetaerina pilula*); April 16, THH+IJC(181), 1♂ (see *Hetaerina titia*); along small creek, April 19, THH+IJC, 1♀.

Brechmorhoga rapax crocosema Ris. Finca La Paz, Buena Vista section above Buena Vista creek in cafetal on steep south facing slope in old part of finca, 4700 to 5000 ft, May 3, THH (234), 1♂.

Erythemis attala (Selys). Tikal, on Uaxactún trail, May 15, THH, 1♂.

This is apparently the first record of this species from the Yucatan Peninsula.

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THE USTILAGINALES (SMUT FUNGI) OF OHIO*

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The smut fungi are in the order Ustilaginales with one family, the Ustilaginaceae, recognized. They are all plant parasites. In recent monographs 276 species in 22 genera are reported in North America and more than 1000 species have been reported from the world (Fischer, 1953; Zundel, 1953; Fischer and Holton, 1957). More than one half of the known smut fungi are pathogens of species in the Gramineae.

Most of the smut fungi are recognized by the black or brown spore masses or sori forming in the inflorescences, the leaves, or the stems of their hosts. The sori may involve the entire inflorescence as *Ustilago nuda* on *Hordeum vulgare* (fig. 2) and *U. residua* on *Danthonia spicata* (fig. 7). *Tilletia foetida*, the cause of bunt of wheat in Ohio, sporulates in the ovularies only and *Ustilago violacea* which has been found in Ohio on *Silene* sp. forms spores only in the anthers of its host.

The sori of *Schizonella melanogramma* on *Carex* (fig. 5) and of *Urocystis anemones* on *Hepatica* (fig. 4) are found in leaves. *Ustilago striiformis* (fig. 6) which causes stripe smut of many grasses has sori which are mostly in the leaves. *Ustilago parlatorei*, found in Ohio on *Rumex* (fig. 3), forms sori in stems, and in petioles and midveins of the leaves.

In a few smut fungi the spore masses are not conspicuous but remain buried in the host tissues. Most of the species in the genera *Entyloma* and *Doassansia* are of this type.

A list of Ohio fungi in the Botany section of Volume 7 (1893) of the *Report of the Geological Survey of Ohio* includes 11 species of smut fungi. A catalogue of Ohio fungi has not been prepared since 1893 (Kellerman and Werner, 1893). The following compilation of Ohio smut fungi includes 45 species in 10 genera on 57 angiosperm hosts. The list is based upon collections in herbaria, reports from the available literature, and numerous collections and observations of the author throughout Ohio. Collections were examined in herbaria of The Ohio State University, the Agricultural Experiment Station, and Oberlin College. Host-fungus reports followed by a literature reference are those based on reports in the literature. Some of these literature reports are based on collections, which were not seen, in the herbarium of the University of Cincinnati (Cooke, 1941).

The nomenclature for the smut fungi is from Fischer's *Manual of the North American Smut Fungi* (1953), and the nomenclature for the host plants is from *Gray's Manual of Botany*, 8th edition.

USTILAGINALES

Cintractia junci (Schw.) Trel.
Juncus tenuis Willd.

Doassansia sagittariae (Westend.) Fisch.
Sagittaria latifolia Willd.
S. sagittifolia L. (Fischer, 1953)

Entyloma australe Speg.
Physalis lanceolata Michx.

Entyloma compositarum Farl.
Ambrosia trifida L.
Rudbeckia laciniata L.

Entyloma eryngii (Cda.) deBary
Sanicula canadensis L.

Entyloma floerkeae Holw.
Floerkea proserpinacoides Willd.

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FIGURE 1. *Cintractia junci* on *Juncus tenuis*.
FIGURE 2. *Ustilago nuda* on *Hordeum vulgare*.
FIGURE 3. *Ustilago parlatorei* in midvein of leaf of *Rumex altissimus*

FIGURE 4. *Urocystis anemones* on *Hepatica acutiloba*.
FIGURE 5. *Schizonella melanogramma* on *Carex pennsylvanica*.
FIGURE 6. *Ustilago striiformis* on *Phleum pratense*.
FIGURE 7. *Ustilago residua* on *Danthonia spicata*.

Entyloma lobeliae Farl.
Lobelia inflata L.

Entyloma menispermi Farl. & Trel.
Menispermum canadense L.

Entyloma nymphaeae (Cunn.) Setch.
Nymphaea odorata Ait.

Melanopsichium pennsylvanicum Hirschh.
Polygonum hydropiperoides Michx.
P. lapathifolium L. (Weiss, 1950–1953)

Schizonella melanogramma (DC.) Schroet.
Carex pennsylvanica Lam. (Fischer, 1953)

Sorosporium cenchri Henn.
Panicum dichotomiflorum Michx.

Sorosporium confusum Jacks.
Aristida dichotoma Michx.

Sorosporium ellisii Wint.
Andropogon scoparius Michx.
A. virginicus L. (Cooke, 1941)

Sphacelotheca hydropiperis (Schum.) deBary
Polygonum sagittatum L.

Sphacelotheca occidentalis (Seym.) Clint.
Andropogon scoparius Michx.

Sphacelotheca reiliana (Kuhn) Clint.
Sorgum vulgare Pers.
Zea mays L.

Sphacelotheca seymouriana Clint.
Andropogon gerardi Vitm.
A. virginicus L.

Sphacelotheca sorghi (Lk.) Clint.
Sorgum vulgare Pers.

Tilletia caries (DC.) Tul.
Triticum aestivum L. (Fischer, 1953)

Tilletia corona Scribn.
Leersia virginica Willd.

Tilletia foetida (Wallr.) Liro
Triticum aestivum L.

Tilletia maclagani (Berk.) Clint.
Panicum virgatum L.

Tilletia pallida G. W. Fisch.
Agrostis canina L. (Fischer, 1953)

Urocystis agropyri (Preuss) Schroet.
Elymus riparius Wieg.

Urocystis anemones (Pers.) Wint.
Anemone quinquefolia L.
Hepatica acutiloba DC.
H. americana (DC.) Ker (Fischer, 1953)

Urocystis carcinodes (Berk. & Curt.) Fisch. v. Waldh.
Cimicifuga racemosa (L.) Nutt.

Urocystis colchici (Schlecht.) Rabenh.
Allium cepa L.
Colchicum autumnale L. (Weiss, 1950–1953)

Urocystis erythronii Clint.
Erythronium americanum Ker (Fischer, 1953)

Urocystis occulta (Wallr.) Rabenh.
Secale cereale L.

Ustilago avenae (Pers.) Rostr.
Arrhenatherum elatius (L.) Mert. & Koch
Avena sativa L.
Hordeum vulgare L.

Ustilago crameri Korn.
Setaria italica (L.) Beauv.

Ustilago heufleri Fckl.
Erythronium albidum Nutt.
E. americanum Ker

Ustilago hordei (Pers.) Lagerh.
Avena sativa L.
Hordeum vulgare L.

Ustilago maydis (DC.) Cda.
Euchlaena mexicana Schrad.
Zea mays L.

Ustilago neglecta Niessl
Setaria glauca (L.) Beauv.
S. viridis (L.) Beauv.

Ustilago nuda (Jens.) Rostr.
Hordeum vulgare L.
Triticum aestivum L.
T. spelta L.

Ustilago oxalidis Ell. & Tracy
Oxalis sp. (Weiss, 1950–1953)

Ustilago parlatorei Fisch. v. Waldh.
Rumex altissimus Wood

Ustilago residua Clint.
Danthonia spicata (L.) Beauv.

Ustilago sphaerogena Burr.
Echinochloa crusgalli (L.) Beauv. (Fischer, 1953)

Ustilago striiformis (Westend.) Niessl
Agrostis tenuis Sibth.
Andropogon virginicus L. (Cooke, 1941)
Calamagrostis canadensis (Michx.) Beauv. (Fischer, 1953)
Dactylis glomerata L.
Phleum pratense L.
Poa pratensis L.

Ustilago syntherismae (Schw.) Pk.
Digitaria sanguinalis (L.) Scop.

Ustilago utriculosa (Nees) Ung.
Polygonum pennsylvanicum L.
P. punctatum Ell. (Cooke, 1941)

Ustilago violacea (Pers.) Roussel
Silene sp.

Key to Ohio Genera of Ustilaginales
Adapted from Fischer and Holton, 1957)

1. Teliospores separate (single) 2
1. Teliospores in groups or balls; rarely single and if so surrounded by an ahdering layer of smaller sterile cells 6
1. Teliospores mostly adhering in pairs; sori forming black rustlike striae in leaves of *Carex* ..... *Schizonella*
2. Sori dusty at maturity 3
2. Sori more or less firmly agglutinated at maturity 5
2. Sori permanently embedded in the host tissues, usually in the leaves, resulting in conspicuous discolored spots; conidia commonly form on the surface of these spots ..... *Entyloma*
3. Teliospores large, 16 μ and larger in diameter; usually in the ovularies ..... *Tilletia*
3. Teliospores mostly 4–18 μ in diameter; in various host parts 4
4. Sori naked or at first covered with a membrane of host cells, usually the epidermis ..... *Ustilago*
4. Sori at first covered with a membrane of fungus cells; in ovularies or other parts of inflorescence ..... *Sphacelotheca*
(*Ustilago* and *Sphacelotheca* are not always considered as distinct.)
5. Teliospores more or less agglutinated and formed around a central columella of host tissue; on Cyperaceae and Juncaceae ..... *Cintractia*
5. Teliospores agglutinated into irregular, hard, gall-like masses consisting of a mixture of host plant tissue and fungus cells; on Polygonaceae ..... *Melanopsichium*
6. Sori embedded in the leaf tissues of the host, resulting in yellowish to brown spots; spore balls with sterile peripheral cells; Ohio species on Alismataceae ..... *Doassansia*
6. Sori dusty or powdery and not permanently embedded in host tissues 7
7. Two to five or six (rarely one) spores in a ball, partially to completely surrounded by smaller, lighter-colored, sterile cells; sori mostly in leaves and stems ..... *Urocystis*
7. Spore balls very fragile at maturity and often disintegrating; without sterile peripheral cells ..... *Sorosporium*
(This genus is difficult to separate from *Ustilago* and *Sphacelotheca* unless young stages are examined when the spore balls are more likely to be intact.)

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AN INTEGRATED CONCEPT OF CARCINOGENIC–ANTICARCINOGENIC ACTION[1]

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Recently, a theory has been formulated (Kovacic, 1960a) which presents a unified picture of the initial step of carcinogenesis, applicable to a wide variety of carcinogens. According to the proposal, cancer originates in the action of an agent, derived from either an artificial carcinogen or a spontaneous process, which directly or indirectly generates hydrogen peroxide in abnormally high concentration. Possible routes whereby such a situation might arise include the inhibition of catalase, catalytic conversion of oxygen to hydrogen peroxide, and decomposition of body water by high energy radiation. Attack by hydrogen peroxide upon the cellular constituents is considered (Kovacic, 1960b) the subsequent stage in the sequence of reactions leading to the cancerous condition. This approach emphasizes the importance of the hydrogen peroxide hypothesis which had been advanced previously by various investigators without wide acceptance. Moreover, for the first time a reasonable theory is provided which correlates in a specific manner a wide array of structurally different carcinogens in relation to the initiation of cancer.

This communication presents an integrated view of carcinogenic-anticarcinogenic action based primarily upon these theoretical considerations, in addition to certain previously reported concepts and investigations.

Three types of cellular response (Haddow, 1947) appear likely in connection with increased levels of hydrogen peroxide. Cellular changes induced by slight increases could be overcome readily in the direction of normal cell regeneration. Exceptionally high concentrations would result in destruction of the cell as a reproductive entity. However, intermediate levels might well effect reversion to a simpler state capable of growth and division—the condition designated as malignancy.

It is likely that the cancer cell is susceptible to attack by a number of different routes. Certain antagonistic agents appear to act as antimetabolites; this category will not be treated in this communication. Anticarcinogens, at least those discussed, will be considered as functioning by either (1) increasing hydrogen peroxide concentration or (2) eliminating excess hydrogen peroxide.

Anticarcinogenic Action by an Increase in Hydrogen Peroxide Concentration

Let us first briefly discuss the mechanism which is proposed (Kovacic, 1960a) for the initiation of cancer, the unifying aspect being the generation of hydrogen

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peroxide. A possible route advanced in the case of aromatic hydrocarbons, aromatic amines, phenols, and azobenzenes entails the conversion of oxygen to hydrogen peroxide through the catalytic action of a metabolite such as an *o*-quinone or a quinonimine. As a more preferred alternative, increases in hydrogen peroxide are considered to result from catalase inhibition by reaction of the carcinogen with enzyme protein accompanied by coordination with iron. Tumor-inducing agents placed in this category include urethan, nitrogen mustards, ethylenimines, ethylene oxides, sulfonic acid esters of diols and *o*-aminophenols, as well as *o*-quinones—a class not yet reported to be carcinogenic. Finally, high energy radiation is known to generate hydrogen peroxide by decomposition of water.

A baffling paradox of oncology exists in the demonstration (Haddow, 1935; Haddow et al., 1948; Greenstein, 1954) that almost all of these same compounds or types of compounds are anticarcinogenic. If the premise is valid that these agents induce cancer by generating excessive amounts of hydrogen peroxide, it may well be that their anticarcinogenic action also is intimately related to the peroxide-forming property. Should this be true, the paradox would be resolved. How might it be possible for increased concentrations of hydrogen peroxide both to induce and combat cancer? An essential component of the interpretation is the thesis advanced by Holman (1956) that ". . . malignant cells—which are already deficient in catalase and abnormally sensitive to oxygen—are so easily killed by overoxidation. . . ." Support for this conclusion was provided by the demonstrated ability of hydrogen peroxide (Hollcroft and Lorenz, 1952; Makino and Tanaka, 1955; Worrall, 1956; Holman, 1957) to destroy cancerous tissue selectively. It is interesting that fetal liver is similar to hepatoma in its extremely low catalase content (Greenstein, 1954) and that agents which are antagonistic to cancer also damage (Skipper, Bennett and Wheeler, 1952) the most actively dividing normal cells. Consistent with the exposition presented thus far is the large reduction (Haddow et al., 1948) in the inhibitory effects of anticarcinogens by use of a high protein diet, as well as the anticancer action (Griffin, Clayton and Baumann, 1949) of the same type of diet, presumably by competition of diet protein with catalase protein for combination with the carcinogen-anticarcinogen.

Since many anticarcinogens also induce cancer, the anticancer property of hydrogen peroxide can be taken as an indication that it may also be carcinogenic. It is significant that hydrogen peroxide is effective in producing melanotic tumors in *Drosophila melanogaster* (Glass and Plaine, 1953; Plaine, 1955a, 1955b; Plaine and Glass, 1955). Similarly, inhibition of neoplasia by 9,10-phenanthrenequinone (Powell, 1951) supports the prediction that *o*-quinones will be added to the list of carcinogenic agents.

Chemotherapy with these various types of compounds, as well as with ionizing radiation, has in general been disappointing—a story of relapse and limited applicability. In light of the theoretical interpretations, this would indicate that increased concentrations of hydrogen peroxide are not completely effective due perhaps to the survival of resistant strains of cancer cells, or else to the generation by peroxide of new malignant cells as the established growths are eliminated. It should follow from this same reasoning that initiation of cancer by hydrogen peroxide would involve a certain degree of simultaneous inhibition. In fact, a number of investigators have observed precisely this type of refractory condition on application of carcinogens (Haddow et al., 1948; see Vesselinovitch, 1958). Numerous other factors involving high degrees of specificity would also be expected to play a role in the carcinogen-anticarcinogen balance.

Anticarcinogenic Action by Elimination of Excess Hydrogen Peroxide

On the basis of the hydrogen peroxide hypothesis, compounds capable of combining readily with hydrogen peroxide or its derived radicals would be classified

as potential cancer preventives. Substances reported as anticarcinogens (Greenstein, 1954; Skipper and Bennett, 1958) which may well function in this manner are mercaptans, maleic acid and aldehydes. Once the neoplastic condition is established, compounds in this category would act only to prevent the further generation of cancerous cells from normal ones. It should be pointed out that these substances or their metabolites can also be included in the alternative proposal which appears to be of wider scope.

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